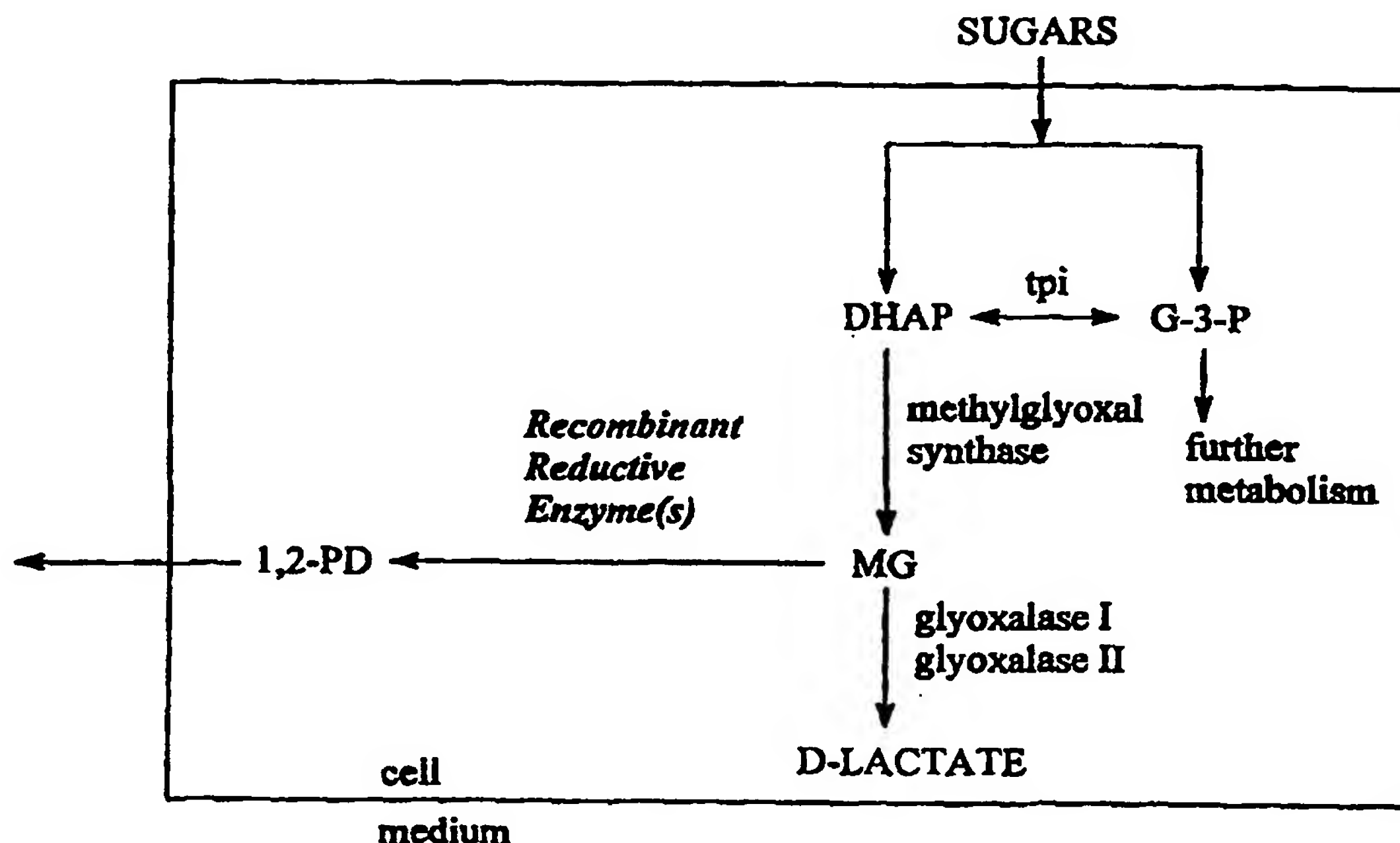




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(54) Title: MICROBIAL PRODUCTION OF 1,2-PROPANEDIOL FROM SUGAR



(57) Abstract

Microorganisms which ferment common sugars into 1,2-propanediol, synthetic operons to effect the transformation, and methods to produce 1,2-propanediol by fermentation of common sugars using the transformed microorganisms are disclosed.

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MICROBIAL PRODUCTION OF 1,2-PROPANEDIOL FROM SUGAR

FIELD OF THE INVENTION

5 The invention is drawn to microorganisms and their use in the production of 1,2-propanediol via microbial fermentation of common sugars. More specifically, the present invention is drawn to recombinant microorganisms having reductive enzyme activity or activities which enable the recombinant microorganism to ferment common sugars to 1,2-propanediol.

BIBLIOGRAPHY

10 Complete bibliographic citations to the references mentioned below are included in the Bibliography section, immediately preceding the Abstract of the Disclosure. Each of the references mentioned below is incorporated herein by reference in its entirety.

DESCRIPTION OF THE PRIOR ART

15 1,2-Propanediol (1,2-PD; also known as propylene glycol) is a major commodity chemical with an annual production greater than one billion pounds in the United States. The major utilization of 1,2-PD is in unsaturated polyester resins, liquid laundry detergents, pharmaceuticals, cosmetics, antifreeze and de-icing formulations.

20 1,2-PD is conventionally produced from petrochemicals. Unfortunately, several toxic chemicals, such as chlorine, propylene oxide, and propylene chlorohydrin are either required or are produced as by-products in the conventional synthesis. In the

conventional route, 1,2-PD is produced by the hydration of propylene oxide, which is obtained from propylene. The synthetic process produces racemic 1,2-PD, an equimolar mixture of the two enantiomers. This chemical process has a number of disadvantages, including the use of large quantities of water to minimize the production of polyglycols. The major problem, however, with the conventional synthetic route to 1,2-PD arises in the production of its intermediate, propylene oxide.

Propylene oxide is manufactured by one of two standard commercial processes: the chlorohydrin process or the hydroperoxide process. The chlorohydrin process involves toxic chlorinated intermediates and the use of caustic or lime. Additionally, this process may result in air emissions of propylene chlorohydrin and chlorine. (*Franklin Associates, Ltd. (1994).*) The hydroperoxide process involves oxidation of propylene by an organic hydroperoxide and results in the stoichiometric co-production of either *tert*-butanol or 1-phenyl ethanol. This make the economics of the production of propylene oxide *via* the hydroperoxide route directly related to the market for the co-produced byproducts. (*Gait (1973).*)

It is known that 1,2-PD is produced by several organisms when grown on exotic sugars. As early as 1937, the fermentation of L-rhamnose to 1,2-PD (later shown to be the S enantiomer) was described by *Kluyver and Schnellen (1937)*. In *E. coli* and a variety of other microorganisms, L-rhamnose and L-fucose are metabolized to L-lactaldehyde and dihydroxyacetone phosphate. (*Sawada and Takagi (1964)* and *Ghalambor and Heath (1962)*, respectively.) Under aerobic conditions, L-lactaldehyde is oxidized in two steps to pyruvate (*Sridhara and Wu (1969)*). Under anaerobic conditions, however, L-lactaldehyde is reduced to S-1,2-PD by a nicotinamide adenine nucleotide (NAD)-linked 1,2-propanediol oxidoreductase (EC 1.1.1.77). The S-1,2-PD produced diffuses into the extra-cellular medium.

Although a variety of microorganisms, including *E. coli*, produce S-1,2-PD from 6-deoxyhexose sugars, *Obradors et al. (1988)*, this route is not commercially feasible because these sugars are extremely expensive. The least expensive of these 6-

deoxyhexose sugars, L-rhamnose, currently sells for approximately \$325 per kilogram (Pfanstiehl Laboratories, Chicago, Illinois).

5 In the mid-1980's, organisms capable of fermenting common sugars, such as glucose and xylose, to R-1,2-PD were discovered. See, for instance, *Tran-Din and Gottschalk* (1985). *Clostridium sphenoides* produces R-1,2-PD via a methylglyoxal intermediate. In this pathway, dihydroxyacetone phosphate (DHAP) is converted to methylglyoxal (MG) by the action of methylglyoxal synthase. The MG is reduced stereospecifically to give D-lactaldehyde. The D-lactaldehyde is then further reduced to give R-1,2-PD. The commercial production of 1,2-PD by *C. sphenoides* is severely
10 limited, however, by the fact it is only produced under phosphate limitation; it is both difficult and expensive to obtain commercial-grade medium components which are free of phosphate. Additionally, only low titers of 1,2-PD are achieved.

15 *Thermoanaerobacterium thermosaccharolyticum* HG-8 (formerly *Clostridium thermosaccharolyticum*, ATCC 31960) also produces R-1,2-PD via methylglyoxal. *Cameron and Cooney* (1986). As with *C. sphenoides*, DHAP is converted to MG. The MG is then reduced at the aldehyde group to yield acetol. The acetol is then further reduced at the ketone group to give R-1,2-PD. For both *C. sphenoides* and *T. thermosaccharolyticum* HG-8, the enzymes responsible for the production of 1,2-PD have not been identified or cloned.

20 SUMMARY OF THE INVENTION

25 The invention is directed to a method of producing 1,2-propanediol by fermentation of sugars. The method comprises culturing a microorganism which expresses one or more enzymes which catalyze production of 1,2-propanediol from intracellular methylglyoxal in a medium containing a sugar carbon source other than a 6-deoxyhexose sugar, whereby the sugar carbon source is metabolized into 1,2-propanediol. Preferably, the method utilizes a recombinant organism containing one or

more recombinant genes whose encoded gene products catalyze the reduction of methylglyoxal to 1,2-propanediol.

5 More specifically, the invention is directed to a method of producing 1,2-propanediol by fermentation with recombinant *E. coli* or yeast which comprises culturing a recombinant *E. coli* or yeast in a medium containing a sugar carbon source selected from the group consisting of arabinose, fructose, galactose, glucose, lactose, maltose, sucrose, xylose, and combinations thereof. The recombinant *E. coli* or yeast includes one or more recombinant genes which encode enzymes selected from the group consisting of aldose reductase, glycerol dehydrogenase, or combinations thereof.

10 The invention is also drawn to a synthetic operon which enables the production of 1,2-propandiol in a microorganism transformed to contain the operon. The operon includes one or more genes whose encoded gene products catalyze the reduction of methylglyoxal to 1,2-PD and a promoter sequence operationally linked to the one or more genes.

15 In a preferred embodiment, the synthetic operon includes at least one promoter sequence, a gene selected from the group consisting of an aldose reductase gene, a glycerol dehydrogenase gene, and combinations thereof; and a gene selected from the group consisting of a methylglyoxal synthase gene, a pyridine nucleotide transferase gene, and combinations thereof, wherein the genes are operationally linked to the promoter.

20 The invention is also drawn to *E. coli* transformed to contain the synthetic operon.

In short, the present invention is drawn to the use of microorganisms, preferably recombinant *E. coli* or *S. cerevisiae*, which express reductive enzyme activity which enables them to produce 1,2-PD, presumably via a reductive pathway leading from methylglyoxal to acetol (or lactaldehyde) to 1,2-PD.

25 If a recombinant microorganism is utilized, the gene sequences encoding the reductive enzyme activity may reside on plasmids within the microorganism, or the gene sequences may be integrated into the chromosome. It is preferred that the recombinant gene sequences be integrated into the genome of the microorganism.

5 The invention utilizes microorganisms which express enzymes which enable the production of 1,2-PD from the fermentation of common sugars. As used herein, the term "common sugars" refers to readily available sugars including, but not limited to, arabinose, fructose, galactose, glucose, lactose, maltose, sucrose, and xylose. Specifically excluded from the term "common sugars" are 6-deoxyhexose sugars such as rhamnose and fucose.

While not being limited to a particular cellular mode of action, it is thought that by properly manipulating enzyme activity, intracellular MG is enzymatically reduced to yield 1,2-PD, which is then secreted into the extracellular environment.

10 The production of MG in the host microorganism can also be simultaneously increased, thereby increasing the production of 1,2-PD. Methylglyoxal production can be maximized by fermenting under phosphate limitation or with the addition of cAMP, as well as by several other methods known to the art. Additionally, selection of suitable host cells, such as methylglyoxal over-producing host cells or mutants which steer metabolism toward the production of 1,2-PD rather than other metabolites, can be utilized.

15 The invention is also drawn to a synthetic operons for transforming a host cell. When incorporated into a host cell, the operon directs the transformed host to produce enzyme activity which converts MG to 1,2-PD and may optionally include genetic elements to increase MG production or to increase the reducing power of the cell. Preferably, the operon includes one or more genes which encode enzymes necessary for expression of aldose reductase activity or glycerol dehydrogenase activity and one or more genes for increased production of MG in the host cell. The operon further includes upstream and/or downstream regulatory elements to control the expression of the gene products(s).

20 The synthetic operon sequence can be incorporated into any number of suitable and well-characterized plasmid vectors for incorporation into prokaryotic or eukaryotic host cells.

25 A major advantage of the present invention is that microbial fermentation provides a clean and "environmentally friendly" synthetic route to 1,2-PD. The microbial process

uses as a substrate a renewable sugar such as glucose or xylose (found in agricultural crops) or lactose (found in dairy industry wastes). Suitable sugars are also produced in commodity amounts from corn and sugar cane and from lignocellulosic biomass.

Also, the microbial process produces no toxic wastes. The byproducts of fermentation are carbon dioxide, alcohols, and organic acids, all of which can be purified as valuable co-products or used as animal feed.

Another distinct advantage of the invention is that it provides a unique route to 1,2-PD from common sugars, a cheap, renewable, and readily available resource.

A further advantage of the present invention is that microbial processes are straightforward to operate and do not involve high temperatures and pressures. Large fermentation facilities such as those used for the production of ethanol can be readily adapted to the production of 1,2-PD.

Another advantage of the invention is that while MG is toxic to cells, by promoting overexpression of recombinant reductase activities, the recombinant cells remain viable and vigorous under conditions that promote MG production. In other words, any potentially toxic excess of MG produced in the recombinant host cell is rapidly converted to 1,2-PD by the recombinant reductase activity (or activities). The 1,2-PD formed is then exported from the cell.

The maximum theoretical yield of 1,2-PD from sugars is favorable: up to 1.5 moles 1,2-PD per mole sugar. And, unlike n-butanol, 1,2-PD itself has very low toxicity to microorganisms. This allows for good cellular growth and viability at high final product titers. Cellular growth at 100 g/L 1,2-PD has been obtained.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic diagram showing the metabolic production of 1,2-PD according to the present invention.

Fig. 2 is a schematic diagram of a preferred repressible transformation vector for use in the present invention, pSE380.

Fig. 3 is an HPLC elution profile of media from recombinant *E. coli* strain AG1 cells which express exogenous aldose reductase activity showing production of 1,2-PD.

Fig. 4 is an HPLC elution profile of a 1,2-PD standard.

Fig. 5 is an HPLC elution profile of media from wild-type *E. coli* showing no production of 1,2-PD.

Fig. 6 is a graph depicting inducible production of 1,2-PD from recombinant *E. coli* containing an operon for the production and regulation of aldose reductase according to the present invention. Aldose reductase production was induced by the addition of IPTG to the culture medium.

Fig. 7 is a graph depicting the inhibition of cell growth due to the presence of 1,2-PD and 1,3-PD. As shown in the graph, 1,2-PD does not result in complete inhibition of cell growth until the amount added to the culture media is approximately 120 g/L.

DETAILED DESCRIPTION OF THE INVENTION

Overview:

An abbreviated schematic diagram of standard sugar metabolism, as well as the pathway for 1,2-PD production according to the present invention, are shown in Fig. 1. In non-transformed *E. coli*, sugars are converted to dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (G-3-P) by glycolytic enzymes common to most organisms. The G-3-P is converted to metabolic byproducts such as ethanol, acetate, and succinate, and is also used for further metabolism.

DHAP is the initial intermediate in the 1,2-PD pathway. DHAP is converted to MG by methylglyoxal synthase. In non-transformed cells, the MG is metabolized to D-lactate as indicated in Fig. 1.

E. coli does not make 1,2-PD from sugars that are readily available. By manipulating various metabolic pathways leading both to and from MG, a microorganism can be made to produce 1,2-PD. While not being limited to any particular mode of

action, it is thought that this reductive conversion takes place in two steps: 1) reduction of MG to acetol or lactaldehyde; and 2) reduction of acetol or lactaldehyde to 1,2-PD. Both reductions can be accomplished by a single enzyme activity or a combination of enzyme activities.

5 The crux of the invention, therefore, is a method to produce 1,2-PD using microorganisms which express enzyme activities whereby the microorganisms convert MG into 1,2-PD. The 1,2-PD so formed may then be harvested from the cell media. The microorganisms can be genetically altered organisms, including mutants or other recombinant strains.

10 The first step of the process is to identify and/or obtain the DNA sequences which encode the desired enzymes and insert or over-express them in the microorganism. This can be accomplished by any means known to the art.

15 For recombinant microorganisms, the preferred enzymes for the production of 1,2-PD are aldose reductase, glycerol dehydrogenase, or both. The preferred form of the aldose reductase gene is rat lens aldose reductase. The preferred form of the glycerol dehydrogenase gene is *E. coli* glycerol dehydrogenase. (In wild-type *E. coli*, glycerol dehydrogenase is regulated to prevent its catalyzing the conversion of MG to 1,2-PD.)

20 It must be noted, however, that because the aldose reductase sequence is highly conserved, the source of the aldose reductase gene is not critical to the present invention. (See, for instance, *Sato et al.* (1995) and *Old et al.* (1990)). Likewise, the source of the glycerol dehydrogenase gene is not critical to the success of the present invention, so long as the gene product displays the required reductive activity. The invention can be successfully practiced with any gene sequence whose expressed gene product provides reductive activity for the conversion of MG to 1,2-PD.

25 The rat lens aldose reductase gene has been cloned and sequenced and is available from the U.S. National Institutes of Health or can be obtained as described in *Sato et al.* and *Old et al., supra*. Other aldose reductase gene sequences are available from "GENBANK" and can be synthesized or sub-cloned using any of several well known methods. Likewise, genes for glycerol dehydrogenase activity are known
30 ("GENBANK").

5 The gene which encodes the enzyme having the required activity is then incorporated into a suitable vector which is used to transform a suitable cellular host. The preferred vector is a plasmid vector. The preferred host is a bacterial host, most preferably *E. coli*, although yeast such as *S. cerevisiae* can be utilized with equal success.

10 Incorporation of the gene into a plasmid transformation or shuttle vector is accomplished by digesting the plasmid with suitable restriction endonucleases, followed by annealing the gene insert to the plasmid "sticky ends," and then ligating the construct with suitable ligation enzymes to re-circulize the plasmid. Each of these steps is well known to those skilled in the art and need not be described in detail here. (See, for instance, *Sambrook, Fritsch, and Maniatis* (1986), *Molecular Cloning, A Laboratory Manual, 2nd Ed.*, incorporated herein by reference for its teaching of vector construction and transformation.)

15 Once successfully transformed with the required gene(s), the recombinant microorganisms produce 1,2-PD from the fermentation of all common sugars, including arabinose, fructose, galactose, glucose, lactose, maltose, sucrose, and xylose. Additionally, it has been shown that fermentation conditions which increase the formation of MG result in increased titers of 1,2-PD.

20 For purposes of this invention, increased MG production in the host cell can be obtained using any method now known or developed in the future. In *E. coli*, methods to obtain increased MG production include, but are not limited to: culturing under low-phosphate (*Ferguson et al.* (1996)), culturing with cyclic AMP and pentoses such as xylose or arabinose (*Ackerman et al.* (1974)), increasing intracellular DHAP (*e.g.* by culturing a triose phosphate isomerase knockout mutant), increasing conversion of DHAP to MG (*e.g.* by over-expressing methylglyoxal synthase), and culturing under unregulated metabolism. (See, for instance, *Freedberg et al.* (1971) and *Kadner et al.* (1992).)

25 Similarly, by utilizing MG over-producing mutants as the host, or by over-expressing endogenous genes (or by introducing exogenous genes) which promote the production of MG, production of 1,2-PD from the transformed cells is maximized.

Careful selection of mutant hosts can also be used to increase the yield of 1,2-PD. *E. coli* mutants, such as AA200 (a triose phosphate isomerase knockout mutant, *E. coli* Genetic Stock Center, New Haven, Connecticut, U.S.A.), can be used as host cells to increase the intracellular levels of MG, thereby increasing 1,2-PD production. Similarly, glyoxalase knockout mutants can also be used as host cells, thereby increasing the intracellular level of MG for conversion to 1,2-PD. Appropriate host selection (using other *E. coli* mutants) also allows the conditions under which 1,2-PD is produced to be varied, e.g., aerobic or anaerobic production, different sugars as a carbon source, etc. For example, when transformed to express exogenous aldose reductase, the *E. coli* strain AA200 noted above has been shown to convert many sugars, including galactose, lactose, and sucrose, into 1,2-PD under aerobic conditions. Analogous transformations can also be accomplished in other host organisms, such as yeast.

Isolation of the 1,2-PD formed from the cell medium can be accomplished by any means known in the separation art. The preferred method is to filter the culture medium to separate cells and cellular debris, and then to isolate the 1,2-PD from the medium by vacuum distillation. (See, for instance, *Simon et al.* (1987).) If so desired, the recombinant microorganisms may be completely lysed by any known means prior to isolation of the 1,2-PD.

***E. coli* Transformed with pKKARX:**

For purposes of brevity and clarity only, the following description is limited to a transformation construct containing an aldose reductase gene. The identical procedure can be followed to insert any gene sequence having the proper activity, such as glycerol dehydrogenase, into a host to thereby enable or maximize the production of 1,2-PD. Other enzymes which promote production of 1,2-PD include: carbonyl reductase (EC 1.1.1.84), glycerol dehydrogenase (EC 1.1.1.6, EC 1.1.1.156), aldehyde reductase (EC 1.1.1.2), methylglyoxal reductase (also known as 2-oxoaldehyde reductase and lactaldehyde dehydrogenase, EC 1.1.1.78), L-glycol dehydrogenase (EC 1.1.1.185), alcohol dehydrogenase EC 1.1.1.1, EC 1.1.1.2), 1,2-PD dehydrogenase, (lactaldehyde

reductase, EC 1.1.1.55), and 1,2-PD oxidoreductase, (lactaldehyde reductase, EC 1.1.1.77).

Any *E. coli* strain can be transformed to contain the aldose reductase insert described herein. The preferred strain is *E. coli* AG1 (*F*⁻, *endA1*, *hsdR17*, {*kn*⁻, *mk*⁺} *supE44*, *thi1*, *recA1*, *gyrA96* *relA1*, λ), available commercially from Stratagene Corporation (La Jolla, California). This strain was used as the host strain for 1,2-PD production in the Examples described below unless otherwise noted. The AA200 and K10 strains were obtained from the *E. coli* Genetic Stock Center (New Haven, Connecticut).

Similarly, any yeast strain can be transformed to contain the desired gene insert. *S. cerevisiae*, numerous strains of which are available from a host of commercial suppliers and the American Type Culture Collection, is preferred.

For transformation of bacteria, a plasmid vector containing the gene insert is preferred. Several suitable vectors are available commercially or can be obtained by methods well known to the art. A preferred expression vector is pKK233-2, available commercially from the Pharmacia Biotech (Piscataway, New Jersey). The sequence of the pKK233-2 vector is shown in SEQ. ID. NO: 1. Suitable restriction enzymes and T4 DNA ligase to manipulate the vector can be obtained from several international suppliers, including Promega Corporation, (Madison, Wisconsin) and New England Biolabs (Beverly, Massachusetts).

The nucleotide sequence of the preferred rat lens aldose reductase gene is shown in SEQ. ID. NO: 3. The amino acid sequence of the encoded aldose reductase enzyme is shown in SEQ. ID. NO: 4.

The aldose reductase gene is inserted into the pKK233-2 plasmid (SEQ. ID. NO: 1) following standard procedures. (This process is essentially identical to that described by *Old et al.* (1990).) The resulting construct is designated pKKARX. The starting pKK233-2 plasmid is designed for direct cloning of eukaryotic genes in *E. coli*. The plasmid contains the highly expressed *trc* promoter (17 base pair spacing between the *trp*-35 region and the *lac* UV5-10 region), the *lacZ* ribosome binding site, and an ATG

initiation codon. To prevent unstable replication, the strong *rrnB* transcription terminator has been introduced downstream of the Multiple Cloning Site. Digestion with *NcoI* exposes the start codon for direct ligation and expression of foreign proteins. Eukaryotic gene fragments lacking a prokaryotic ribosome binding site and/or an ATG can be inserted in the correct reading frame by using one of several commercially available *NcoI* linkers. (Available, for instance, from Pharmacia Biotech, Piscataway, New Jersey). The *NcoI* recognition sequence, CCATGG, commonly occurs at the initiation codon of eukaryotic genes, allowing direct ligation to the vector.

E. coli can then be transformed using the pKKARX construct. All transformations described herein were performed by the calcium chloride method using standard and well-known methodologies. While the calcium chloride method is preferred, transformation can be accomplished with equal success using any of several conventional procedures, such as electroporation and the like.

Once transformed with pKKARX, wild-type *E. coli* host cells produce 1,2-PD from arabinose, glucose, and xylose. Analysis for production of 1,2-PD is performed as described in Example 1, below.

E. coli Transformed with pSEARX:

Another aspect of the invention is to transform the host with an insert which includes inducible or repressible genetic elements. This allows the production of 1,2-PD to be switched on or off by addition of a suitable inducer or repressor.

The preferred construct, designated pSEARX, is constructed by digesting pKKARX (described above) and a commercially-available vector designated pSE380 (Invitrogen, La Jolla, California) with *NcoI* and *EcoRI*. The resulting fragments from *NcoI* and *EcoRI* digestion are then separated by agarose gel electrophoresis, and the aldose reductase gene and pSE380 vector purified using "GENECLEAN" (Bio 101 Inc., La Jolla, California) according to the manufacturer's instructions. The two fragments are then ligated and transformed into AG1 using standard procedures (*Sambrook et al., supra*).

5 A schematic of the starting pSE380 plasmid is shown in Fig. 2. The pSE380 plasmid includes a strong *trc* promoter for high level transcription, as well as the *lacO* operator and *lacI^q* repressor gene (which allows transcriptional regulation in any *E. coli* strain). While the pSE380 starting plasmid is preferred, any construct containing an inducible or repressible promoter which can control the expression of gene sequences operationally linked to the promoter will function with equal success. In addition to the *trc* promoter, examples of well known promoters which can be utilized include *lac*, *tac*, and *phoA*. The nucleotide sequence of pSE380 is given in SEQ. ID. NO: 2.

10 Inducing a wild-type *E. coli* host transformed with pSEARX by adding IPTG to the media results in the production of 1,2-PD when the host is grown on arabinose, glucose, and xylose.

Mutant host selection to maximize utilizable substrates and 1,2-PD production:

15 Increased flexibility when producing 1,2-PD from transformed *E. coli* or yeast is afforded by selection of a suitable mutant host. For instance, when transformed with either pKKARX or pSEARX as described above, triose phosphate isomerase knockout mutant bacteria, such as *E. coli* strain AA200, produce 1,2-PD when fermented with any combination of arabinose, galactose, glucose, lactose, sucrose, and xylose. Triose phosphate isomerase catalyzes the interconversion of DHAP to G-3-P. (See Fig. 1.) By
20 utilizing a host mutant which lacks triose phosphate isomerase activity, the metabolic fate of DHAP is directed to the formation of MG, which is then converted by various reductive enzyme activities into 1,2-PD, thereby increasing 1,2-PD titers.

25 Likewise, 1,2-PD production can be maximized by utilizing other mutants lacking one or more enzymes which decrease intracellular pools of MG. For instance, the normal metabolic pathway to detoxify intracellular methylglyoxal utilizes glyoxalase I. Glyoxalase I catalyzes the conversion of MG to S-D-lactoylglutathione, which is subsequently converted to lactate by glyoxalase II. Consequently, when a host is transformed to express a recombinant enzyme having MG reducing activity, the MG-reducing enzyme competes with glyoxalase I for the available MG. By utilizing

glyoxalase I knockout mutants, the intracellular pool of MG for conversion to 1,2-PD is increased, and the ultimate production of 1,2-PD is likewise increased.

Glyoxalase mutants can be constructed in *E. coli*, yeast, or any other suitable host, using standard techniques. Because several glyoxalase oligonucleotide sequences are known ("GENBANK"), the most straightforward route to obtain a glyoxalase mutant is to recombine a deletion into the chromosomal copy of the glyoxalase gene whereby glyoxalase activity is destroyed. An example of how this can be done is described in *Koob et al.* (1994).

Negative Controls

To provide a negative control for the pKKARX and pSEARX constructs, a plasmid designated pKKARX/*Pst*I was constructed by digesting pKKARX with *Pst*I and purifying the vector portion of the resulting digest. The vector was then self-ligated resulting in an approximately 1 kb deletion within the aldose reductase gene on the plasmid. AG1 cells transformed with pKKARX/*Pst* show no aldose reductase activity or 1,2-PD production.

Yeast Hosts:

In an analogous fashion, yeast (as well as other cellular hosts) can be transformed to contain the aldose reductase gene (or any of the other genes listed above) and can be used to produce 1,2-PD by fermentation of common sugars.

In yeast, the aldose reductase gene is first inserted into an appropriate shuttle vector. In the preferred embodiment, an aldose reductase cassette is ligated into YpJ66 digested with *Eco*RI/*Kpn*I, thus replacing the *galK* cassette with an aldose reductase cassette between *Eco*RI and *Kpn*I. YpJ66 is constructed from YEp352, whose oligonucleotide sequence is shown in SEQ. ID. NO: 5., and can be constructed according to the method of *Hill et al.* (1986). In short, this is accomplished by inserting the CUP1 promoter, (*galK*) and CYC1 terminator sequence into the *Xba*I site of Yep352.

Preferably, the vector is then transformed into YPH500 (ATCC 76626) (*leu⁻*, *trp⁻*, *ura⁻*, *lys⁻*, *ade⁻*, *his⁻*) by standard methods and fed the required amino acids for growth, except uracil, which is used as the marker to maintain the plasmid in yeast. In the same fashion as transformed *E. coli*, yeast transformed to contain the aldose reductase insert produce 1,2-PD in isolatable quantities when fermented on a wide variety of common sugars, including galactose, glucose, sucrose, fructose, and maltose.

Other genetically altered strains can produce 1,2-PD when cultured on other sugar carbon sources such as xylose and lactose.

Synthetic Operons for the Production of 1,2-PD:

Ideally, three criteria should be maximized in order to maximize production of 1,2-PD. These three criteria are: increased production of MG, increased production of enzymes to convert MG to 1,2-PD, and increased production of enzymes such as pyridine nucleotide transferase to increase the reducing power within the cell (and thereby favor the reduction of MG to 1,2-PD). In this embodiment of the invention, a methylglyoxal synthase gene for increasing production of MG, and/or an aldose reductase or glycerol dehydrogenase gene for converting MG to 1,2-PD, and/or a pyridine nucleotide transferase gene for increasing the reductive power of the host cell are operationally linked, in any order, under the control of one or more promoters, to yield a synthetic operon which maximizes the production of 1,2-PD in host microorganisms transformed with the operon.

The methylglyoxal synthase gene has been cloned and expressed in *E. coli* and is shown in SEQ. ID. NO: 6. The ATG initiation codon is underlined. (See also *Percy and Harrison* (1996)). Likewise, the pyridine nucleotide transferase gene, encoding subunits A and B, is also known and is shown in SEQ. ID. NO: 7. The amino acid sequences of the encoded A and B subunits of pyridine nucleotide transferase are shown in SEQ. ID. NO: 8 and SEQ. ID. NO: 9, respectively. The glycerol dehydrogenase gene has also been identified; its oligonucleotide sequence is shown in SEQ. ID. NO: 10. The glycerol dehydrogenase amino acid sequence is shown in SEQ. ID. NO: 11.

To construct the synthetic operon according to the present invention, SEQ. ID. NO: 3 (aldose reductase), SEQ. ID. NO: 6 (methylglyoxal synthase), SEQ. ID. NO: 7 (pyridine nucleotide transferase) and/or SEQ. ID. NO: 10 (glycerol dehydrogenase) are operatively linked together in a 5' to 3' orientation. The order of the genes is not critical to the functionality of the operon, so long as each gene is operationally linked to its neighbor in a 5' to 3' orientation.

The gene sequences are inserted into a suitable plasmid host which includes one or more promoter sequences such that the promoter is operationally linked to the gene sequences and can function to promote or repress transcription of the genes. Suitable promoter sequences include any number of well known and widely used promoters such as *lac*, *trc*, *tac*, and *phoA*. For instance, pSE380 contains the *trc* promoter. A very large number of suitable transformation vectors containing the above-listed promoters are commercially available from several international suppliers.

The gene insert containing the functional genes is constructed by standard and well known means. In short, the individual gene inserts are digested with an appropriate restriction enzyme to yield complimentary "sticky ends," which are then annealed to one another and ligated with T4 ligase. The gene construct is then again digested to yield appropriate complimentary ends to be operationally inserted into a plasmid vector containing the promoter sequences. Many commercial plasmids contain a Multiple Cloning Site which allows any number of different restriction enzymes to be utilized to effect insertion of the construct into the plasmid vector. The vector is then used to transform a suitable host, as described above.

When transformed with the synthetic operon as described herein, the recombinant microorganism produces 1,2-PD in isolatable quantities.

The synthetic operon need not contain any or all of the above-noted genes. At a minimum, at least one gene encoding an enzyme to effect the reduction of MG to 1,2-PD must be present, such as the aldose reductase gene or the glycerol dehydrogenase gene or some other gene or genes. In addition, either or both of the methylglyoxal synthase and pyridine nucleotide transferase genes may be present. Additionally, the

genes need not all be under the control of a single promoter. For purposes of flexibility, each individual gene can be placed under the control of a separate promoter.

5 Additionally, an alternative to utilizing a triose phosphate isomerase knockout mutant host strain is to place the triose phosphate isomerase gene under the control of a promoter sequence. This enables transcription of the gene to be switched on or off, depending upon the conditions present. To effect insertion of promoter sequence in operational orientation to the triose phosphate isomerase gene, standard recombinant genetic techniques are utilized. (Again, see *Sambrook, Fritsch, and Maniatis (1986), Molecular Cloning, A Laboratory Manual, 2nd Ed.*) The promoter of interest is placed
10 into a suitable vector, preferably a plasmid vector, which contains appropriate cloning sequences to enable operational insertion of the promoter sequence into the genome of the host organism. Successful incorporation of the plasmid is determined via antibiotic resistance and/or testing for induction (or repression) of triose phosphate isomerase. Such method are well known to those skilled in the art.

15 EXAMPLES

The following Examples are included solely for illustrative purposes to provide a more complete understanding of the invention. The Examples do not limit the scope
20 of the invention disclosed or claimed herein in any fashion.

EXAMPLE 1: Chromatographic Analysis of Culture Broth

25 Figs. 3 and 5 depict HPLC analyses of the culture broth of an *E. coli* strain AG1 transformed to express aldose reductase (using pKKARX) and a non-transformed culture of the same strain, respectively. Figure 4 depicts an HPLC elution profile of a 1,2-PD standard solution. With reference to Fig. 3 and 5, the fermentations were performed under standard anaerobic conditions using 5 g/L glucose as carbon source. Media samples were centrifuged and filtered before analysis.

30 To generate the plots shown in Figs. 3 and 5, an organic acids column (Bio-Rad "HPX87H", Hercules, California) was used to quantify 1,2-PD, ethanol, sugars, and

organic acids under the following conditions: 50 μ L sample size, pH 2 (H_2SO_4);, 0.5 mL/min flow rate, and 40°C column temperature. Peaks were detected by a refractive index detector at 40°C.

5 The 1,2-PD peak from the organic acids column was further analyzed by injection onto a cation-exchange column (Waters "SUGAR-PAK II," Marlboro, Massachusetts). The 1,2-PD peak isolated from the fermentation broth elutes at exactly the same time as the 1,2-PD control. The secondary peak identifications were performed on the "SUGAR-PAK II" column under the following conditions: 50 μ L sample size, Milli-Q water mobile phase; 0.5 mL/min flow rate; and 90°C column temperature.

10 Additionally, analyses were performed in which the 1,2-PD peak from the organic acids column was collected and subjected to gas chromatographic (GC) analysis and mass spectrographic analysis. The GC peak co-eluted with the 1,2-PD standard. Mass spectrometry showed the same fragmentation pattern as the 1,2-PD standard. The fact that the same peak co-eluted with a 1,2-PD standard on 3 different columns (HPLC organic acids column, HPLC sugars column, and GC), with different methods of separation, as well as its fragmentation in mass spectrography, its identification as 1,2-PD is quite certain.

20 **EXAMPLE 2: Production of 1,2-Propanediol from Various Common Sugars**

25 In this Example, a triose phosphate isomerase mutant (*tpi*-), AA200, was transformed with pSEARX containing the gene for aldose reductase as described above. (This transformed cell line is designated AA200::pSEARX). The non-transformed AA200 mutant yields higher intracellular concentrations of methylglyoxal, the precursor to 1,2-PD, than the wild-type. (See *Hopper and Cooper* (1972).) When transformed with pSEARX, the AA200::pSEARX cell line produced 1,2-PD from arabinose, galactose, glucose, lactose, sucrose, and xylose. The yield of 1,2-PD from AA200::pSEARX fermented with various sugars was as follows:

Table 1

<u>SUGAR</u>	<u>TITER 1,2-PD, mg/L</u>
Galactose	66
Glucose	71
Lactose	6
Sucrose	7
Xylose	49

Fermentation was performed using standard anaerobic fermentation procedures using 10 g/L of the appropriate sugar. The fermentation was allowed to proceed for 24 hours prior to analysis for 1,2-PD.

EXAMPLE 3: Inducible production of 1,2-PD

In this Example, the results of which are depicted in Fig. 6, *E. coli* strain AG1 was transformed as described herein with the pSEARX plasmid containing the aldose reductase gene. The transformed cells were then cultured under standard anaerobic conditions on 5 g/L glucose with increasing levels of the promoter IPTG. The X-axis of Fig. 6 gives the concentration of IPTG in millimolarity. The right-hand Y-axis (■) reports the production of 1,2-PD in mg/L as a function of IPTG concentration. Likewise the left-hand Y-axis (●) reports the activity of aldose reductase in U/mg. As is clearly shown in Fig. 6, inducing the promoter leads to the production of 1,2-PD.

EXAMPLE 4: Inhibition of Cell Growth by 1,2-PD

Here, an Experiment was performed to determine at what level the presence 1,2-PD and 1,3-PD begin to have an adverse effect on *E. coli* cell growth. Anaerobic batch cultivations of *E. coli* were carried out in 10 mL culture tubes. Nine different batch cultivations, covering a range between 0 and 120 g/L of 1,2-PD (○) and 1,3-propanediol (□) were carried out in triplicate. Using optical density measurements, the growth in each tube was monitored and the specific growth rate determined. The results are depicted in Fig. 7. The ratio μ/μ_0 has been plotted as a function of the concentration of 1,2-PD and 1,3-PD (I, g/L). The value of μ equals the specific growth rate determined for the corresponding concentration of 1,2 or 1,3-PD; the value of μ_0 equals

the specific growth rate determined in the absence of any 1,2-PD or 1,3-PD. The error bars indicate the standard deviation between the triplicate experiments. As can be seen from Fig. 7, 1,2-PD does not cause complete inhibition of cell growth until a concentration of approximately 120 g/L is reached.

EXAMPLE 5: Anaerobic Production of 1,2-PD Utilizing Recombinant Glycerol Dehydrogenase Gene

E. coli strain AG1 was transformed in standard fashion with pSE380 containing a gene for *E. coli* glycerol dehydrogenase. The plasmid, designated pNEA10, was constructed in standard fashion. The transformed cells were then cultured under strictly anaerobic conditions on 10 g/L glucose. The fermentation was allowed to proceed for 12 hours to allow cell growth prior to addition of IPTG. The fermentation was then allowed to proceed for an additional 24 hours prior to analysis for 1,2-PD. The results are shown in Table 2:

Table 2

plasmid ——	IPTG <u>mM</u>	1,2-PD Titer <u>mg/L</u>	Activity* <u>U/mg</u>
pSE380†	0.0	0	0.10
pNEA10	0.0	100	0.48
pNEA10	0.05	190	3.00
pNEA10	0.10	220	2.70
pNEA10	0.25	220	3.10

*measured using glycerol as a substrate

†control plasmid without glycerol dehydrogenase gene

EXAMPLE 6: Production of 1,2-PD by Host Containing Recombinant Glycerol Dehydrogenase Gene in Combination With Promoter

E. coli strain AG1 was transformed as described in Example 5. The transformed cells were then cultured on 15 g/L glucose under anaerobic conditions. Prior to the fermentation, the media was not purged of oxygen. IPTG was added at the start of the

fermentation. The fermentation was allowed to proceed for 36 hours prior to analysis for 1,2-PD. The results are depicted in Table 3:

Table 3

plasmid ——	IPTG <u>mM</u>	1,2-PD Titer <u>mg/L</u>	Activity* <u>U/mg</u>
pSE380†	0.0	0	0.10
pNEA10	0.0	30	2.31
pNEA10	0.05	100	9.89

*measured using acetol as a substrate

†control plasmid without glycerol dehydrogenase gene

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Cameron, Douglas C.
Shaw, Anita J.
Altaras, Nedim E.
- (ii) TITLE OF INVENTION: MICROBIAL PRODUCTION OF
1,2-PROPANEDIOL FROM SUGAR
- (iii) NUMBER OF SEQUENCES: 11
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: DeWitt Ross & Stevens S.C.
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 - (C) CITY: Madison
 - (D) STATE: WI
 - (E) COUNTRY: U.S.A.
 - (F) ZIP: 53717-1914
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Sara, Charles S.
 - (C) REFERENCE/DOCKET NUMBER: 09820.037
- (ix) TELECOMMUNICATION INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 4593 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Vector pKK232-2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4476 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Vector pSE380

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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GCTTGAGCG AACGACCTAC ACCGAACTGA GATACCTACA GCGTGAGCTA TGAGAAAGCG	2580
CCACGCTTCC CGAAGGGAGA AAGGCGGACA GGTATCCGGT AAGCGGCAGG GTCGGAACAG	2640
GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA TCTTTATAGT CCTGTCGGGT	2700
TTCGCCACCT CTGACTTGAG CGTCGATTTT TGTGATGCTC GTCAGGGGGG CGGAGCCTAT	2760
GGAAAAACGC CAGCAACGCG GCCTTTTTTAC GGTTCCTGGC CTTTTGCTGG CCTTTTGCTC	2820
ACATGTTCTT TCCTGCGTTA TCCCCTGATT CTGTGGATAA CCGTATTACC GCCTTTGAGT	2880
GAGCTGATAC CGCTCGCCGC AGCCGAACGA CCGAGCGCAG CGAGTCAGTG AGCGAGGAAG	2940
CGGAAGAGCG CCTGATGCGG TATTTTCTCC TTACGCATCT GTGCGGTATT TCACACCGCA	3000
TATGGTGCAC TCTCAGTACA ATCTGCTCTG ATGCCGCATA GTTAAGCCAG TATACACTCC	3060
GCTATCGCTA CGTGACTGGG TCATGGCTGC GCCCCGACAC CCGCCAACAC CCGCTGACGC	3120
GCCCTGACGG GCTTGTCTGC TCCCGGCATC CGCTTACAGA CAAGCTGTGA CCGTCTCCGG	3180

GAGCTGCATG TGTCAGAGGT TTTCACCGTC ATCACCGAAA CGCGCGAGGC AGCAGATCAA	3240
TTTCGCGCGCG AAGGCGAAGC GGCATGCATT TACGTTGACA CCATCGAATG GCGCAAAACC	3300
TTTCGCGGTA TGGCATGATA GCGCCCGGAA GAGAGTCAAT TCAGGGTGGT GAATGTGAAA	3360
CCAGTAACGT TATACGATGT CGCAGAGTAT GCCGGTGTCT CTTATCAGAC CGTTTCCCGC	3420
GTGGTGAACC AGGCCAGCCA CGTTTCTGCG AAAACGCGGG AAAAAGTGGA AGCGGCGATG	3480
GCGGAGCTGA ATTACATTCC CAACCGCGTG GCACAACAAC TGGCGGGCAA ACAGTCGTTG	3540
CTGATTGGCG TTGCCACCTC CAGTCTGGCC CTGCACGCGC CGTCGCAAAT TGTCGCGGCG	3600
ATTAAATCTC GCGCCGATCA ACTGGGTGCC AGCGTGGTGG TGTCGATGGT AGAACGAAGC	3660
GGCGTCGAAG CCTGTAAAGC GGCGGTGCAC AATCTTCTCG CGCAACGCGT CAGTGGGCTG	3720
ATCATTAAT ATCCGCTGGA TGACCAGGAT GCCATTGCTG TGGAAGCTGC CTGCACTAAT	3780
GTTCCGGCGT TATTTCTTGA TGTCTCTGAC CAGACACCCA TCAACAGTAT TATTTTCTCC	3840
CATGAAGACG GTACGCGACT GGGCGTGGAG CATCTGGTCC CATTGGGTCA CCAGCAAATC	3900
GCGCTGTTAG CGGGCCCAT T AAGTTCTGTC TCGGCGCGTC TCGCTCTGGC TGGCTGGCAT	3960
AAATATCTCA CTCGCAATCA AATTCAGCCG ATAGCGGAAC GGGGAAGGCGA CTGGAGTGCC	4020
ATGTCCGGTT TTCAACAAAC CATGCAAATG CTGAATGAGG GCATCGTTCC CACTGCGATG	4080
CTGGTTGCCA ACGATCAGAT GGCCTGGGC GCAATGCGCG CCATTACCGA GTCCGGGCTG	4140
CGCGTTGGTG CGGATATCTC GGTAGTGGGA TACGACGATA CCGAAGACAG CTCATGTTAT	4200
ATCCCGCCGT TAACCACCAT CAAACAGGAT TTTCGCCTGC TGGGGCAAAC CAGCGTGGAC	4260
CGCTTGCTGC AACTCTCTCA GGGCCAGGCG GTGAAGGGCA ATCAGCTGTT GCCCGTCTCA	4320
CTGGTGAAAA GAAAAACCAC CCTGGCGCCC AATACGCAA CCGCCTCTCC CCGCGCGTTG	4380
GCCGATTCAT TAATGCAGCT GGCACGACAG GTTTCCCGAC TGGAAAGCGG GCAGTGAGCG	4440
CAACGCAATT AATGTGAGTT AGCGCGAATT GATCTT	4476

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1337 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Rat lens aldose reductase gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTCTTGCGGG TCGTTGTGCG TAACTTGCAG CAATCATGGC TAGCCATCTG GAACTCAACA	60
ACGGCACCAA GATGCCCACC CTGGGTCTGG GCACCTGGAA GTCTCCTCCT GGCCAGGTGA	120
CCGAGGCTGT GAAGGTTGCT ATCGACATGG GGTATCGCCA CATTGACTGC GCCCAGGTGT	180
ACCAGAATGA GAAGGAGGTG GGGGTGGCCC TCCAGGAGAA GCTCAAGGAG CAGGTGGTGA	240
AGCGCCAGGA TCTCTTCATT GTCAGCAAGC TGTGGTGCAC GTTCCACGAC CAGAGCATGG	300
TGAAAGGGGC CTGCCAGAAG ACGCTGAGCG ACCTGCAGCT GGACTACCTG GACCTCTACC	360
TTATTCACTG GCCAACTGGC TTCAAGCCTG GGCCTGACTA TTTCCCCCTG GATGCATCGG	420
GAAACGTGAT TCCTAGTGAC ACCGATTTTG TGGACACTTG GACGGCTATG GAGCAACTAG	480
TGGATGAAGG TTTGGTAAAA GCAATCGGAG TCTCCAACCT CAACCCTCTT CAGATTGAGA	540
GGATCTTGAA CAAACCTGGC TTAAAGTATA AGCCTGCTGT TAACCAGATC GAGTGCCACC	600
CATACCTAAC TCAGGAGAAG CTGATTGAGT ACTGCCATTG CAAAGGCATC GTGGTGA CTG	660
CATACAGTCC CCTTG GTTCT CCTGACAGGC CCTGGGCCAA GCCTGAGGAC CCCTCTCTCC	720
TGGAGGATCC CAGGATCAAG GAAATTGCAG CCAAGTACAA TAAAACTACA GCCCAGGTGC	780
TGATCCGGTT CCCCATCCAA AGGAACCTGG TCGTGATCCC CAAGTCTGTG ACACCAGCAC	840
GTATTGCTGA GAACTTTAAG GTCTTTGACT TTGAGCTGAG CAATGAGGAC ATGGCCACTC	900
TACTCAGCTA CAACAGGAAC TGGAGGGTGT GCGCCTTGAT GAGCTGTGCC AAACACAAGG	960
ATTACCCCTT CCACGCAGAA GTCTGAAGCT GTGGTGGACG AATCCTGCTC CTCCCCAAGC	1020
GACTTAACAC ATGTTCTTTC TGCCTCATCT GCCCTTGCAA GTGTCCCTCT GCACTGGGTG	1080
GCACCTTGCA GACCAGATGG TGAGAGTTTG TTAGTTTGAC GTAGAATGTG GAGGGCAGTA	1140
CCAGTAGCTG AGGAGTTTCT TCGGCCTTTC TTGGTCTTCT TCCCACCTGG AGGACTTTAA	1200
CACGAGTACC TTTTCCAACC AAAGAGAAAG CAAGATTTAT AGCCCAAGTC ATGCCACTAA	1260
CACTTAAATT TGAGTGCTTA GAACTCCAGT CCTATGGGGG TCAGACTTTT TGCCTCAAAT	1320

AAAAACTGCT TTTGTCG

1337

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 316 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Rat lens aldose reductase

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Ala	Ser	His	Leu	Glu	Leu	Asn	Asn	Gly	Thr	Lys	Met	Pro	Thr	Leu	1	5	10	15
Gly	Leu	Gly	Thr	Trp	Lys	Ser	Pro	Pro	Gly	Gln	Val	Thr	Glu	Ala	Val	20	25	30	
Lys	Val	Ala	Ile	Asp	Met	Gly	Tyr	Arg	His	Ile	Asp	Cys	Ala	Gln	Val	35	40	45	
Tyr	Gln	Asn	Glu	Lys	Glu	Val	Gly	Val	Ala	Leu	Gln	Glu	Lys	Leu	Lys	50	55	60	
Glu	Gln	Val	Val	Lys	Arg	Gln	Asp	Leu	Phe	Ile	Val	Ser	Lys	Leu	Trp	65	70	75	80
Cys	Thr	Phe	His	Asp	Gln	Ser	Met	Val	Lys	Gly	Ala	Cys	Gln	Lys	Thr	85	90	95	
Leu	Ser	Asp	Leu	Gln	Leu	Asp	Tyr	Leu	Asp	Leu	Tyr	Leu	Ile	His	Trp	100	105	110	
Pro	Thr	Gly	Phe	Lys	Pro	Gly	Pro	Asp	Tyr	Phe	Pro	Leu	Asp	Ala	Ser	115	120	125	
Gly	Asn	Val	Ile	Pro	Ser	Asp	Thr	Asp	Phe	Val	Asp	Thr	Trp	Thr	Ala	130	135	140	
Met	Glu	Gln	Leu	Val	Asp	Glu	Gly	Leu	Val	Lys	Ala	Ile	Gly	Val	Ser	145	150	155	160
Asn	Phe	Asn	Pro	Leu	Gln	Ile	Glu	Arg	Ile	Leu	Asn	Lys	Pro	Gly	Leu	165	170	175	
Lys	Tyr	Lys	Pro	Ala	Val	Asn	Gln	Ile	Glu	Cys	His	Pro	Tyr	Leu	Thr	180	185	190	
Gln	Glu	Lys	Leu	Ile	Glu	Tyr	Cys	His	Cys	Lys	Gly	Ile	Val	Val	Thr	195	200	205	
Ala	Tyr	Ser	Pro	Leu	Gly	Ser	Pro	Asp	Arg	Pro	Trp	Ala	Lys	Pro	Glu	210	215	220	

Asp Pro Ser Leu Leu Glu Asp Pro Arg Ile Lys Glu Ile Ala Ala Lys
 225 230 235 240
 Tyr Asn Lys Thr Thr Ala Gln Val Leu Ile Arg Phe Pro Ile Gln Arg
 245 250 255
 Asn Leu Val Val Ile Pro Lys Ser Val Thr Pro Ala Arg Ile Ala Glu
 260 265 270
 Asn Phe Lys Val Phe Asp Phe Glu Leu Ser Asn Glu Asp Met Ala Thr
 275 280 285
 Leu Leu Ser Tyr Asn Arg Asn Trp Arg Val Cys Ala Leu Met Ser Cys
 290 295 300
 Ala Lys His Lys Asp Tyr Pro Phe His Ala Glu Val
 305 310 315

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5181 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Yeast shuttle vector YEp352

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATGACCATGA TTACGAATTC GAGCTCGGTA CCCGGGGATC CTCTAGAGTC GACCTGCAGG 60
 CATGCAAGCT TGGCACTGGC CGTCGTTTCA CAACGTCGTG ACTGGGAAAA CCCTGGCGTT 120
 ACCCAACTTA ATCGCCTTGC AGCACATCCC CCCTTCGCCA GCTGGCGTAA TAGCGAAGAG 180
 GCCCGCACCG ATCGCCCTTC CCAACAGTTG CGCAGCCTGA ATGGCGAATG GCGCCTGATG 240
 CGGTATTTTC TCCTTACGCA TCTGTGCGGT ATTTACACACC GCATAGGGTA ATAAGTGATA 300
 TAATTAAATT GAAGCTCTAA TTTGTGAGTT TAGTATACAT GCATTTACTT ATAATACAGT 360
 TTTTGTAGTTT TGCTGGCCGC ATCTTCTCAA ATATGCTTCC CAGCCTGCTT TTCTGTAACG 420
 TTCACCCTCT ACCTTAGCAT CCCTTCCCTT TGCAAATAGT CCTCTTCCAA CAATAATAAT 480
 GTCAGATCCT GTAGAGACCA CATCATCCAC GGTTCCTATAC TGTTGACCCA ATGCGTCTCC 540
 CTTGTCATCT AAACCCACAC CGGGTGTCAT AATCAACCAA TCGTAACCTT CATCTCTTCC 600
 ACCCATGTCT CTTTGAGCAA TAAAGCCGAT AACAAAATCT TTGTCGCTCT TCGCAATGTC 660
 AACAGTACCC TTAGTATATT CTCCAGTAGA TAGGGAGCCC TTGCATGACA ATTCTGCTAA 720
 CATCAAAAGG CCTCTAGGTT CCTTTGTTAC TTCTTCTGCC GCCTGCTTCA AACCGCTAAC 780

AATACCTGGG CCCACCACAC CGTGTGCATT CGTAATGTCT GCCCATTCTG CTATTCTGTA	840
TACACCCGCA GAGTACTGCA ATTTGACTGT ATTACCAATG TCAGCAAATT TTCTGTCTTC	900
GAAGAGTAAA AAATTGTACT TGGCGGATAA TGCCTTTAGC GGCTTAACTG TGCCCTCCAT	960
GGAAAAATCA GTCAAGATAT CCACATGTGT TTTTAGTAAA CAAATTTTGG GACCTAATGC	1020
TTCAACTAAC TCCAGTAATT CCTTGGTGGT ACGAACATCC AATGAAGCAC ACAAGTTTGT	1080
TTGCTTTTTCG TGCATGATAT TAAATAGCTT GGCAGCAACA GGACTAGGAT GAGTAGCAGC	1140
ACGTTCCCTTA TATGTAGCTT TCGACATGAT TTATCTTCGT TTCGGTTTTT GTTCTGTGCA	1200
GTTGGGTAA GAATACTGGG CAATTTTCATG TTTCTTCAAC ACTACATATG CGTATATATA	1260
CCAATCTAAG TCTGTGCTCC TTCCTTCGTT CTTCTTCTG TTCGGAGATT ACCGAATCAA	1320
AAAAATTTCA AAGAAACCGA AATCAAAAAA AAGAATAAAA AAAAAATGAT GAATTGAAAA	1380
GCTCTTGTTA CCCATCATTG AATTTTGAAC ATCCGAACCT GGGAGTTTTT CCTGAAACAG	1440
ATAGTATATT TGAACCTGTA TAATAATATA TAGTCTAGCG CTTTACGGAA GACAATGTAT	1500
GTATTTCCGT TCCTGGAGAA ACTATTGCAT CTATTGCATA GGTAATCTTG CACGTCGCAT	1560
CCCCGGTTCA TTTTCTGCGT TTCCATCTTG CACTTCAATA GCATATCTTT GTTAACGAAG	1620
CATCTGTGCT TCATTTTGTA GAACAAAAAT GCAACGCGAG AGCGCTAATT TTTCAAACAA	1680
AGAATCTGAG CTGCATTTTT ACAGAACAGA AATGCAACGC GAAAGCGCTA TTTTACCAAC	1740
GAAGAATCTG TGCTTCATTT TTGTAAAACA AAAATGCAAC GCGAGAGCGC TAATTTTTCA	1800
AACAAAGAAT CTGAGCTGCA TTTTACAGA ACAGAAATGC AACGCGAGAG CGCTATTTTA	1860
CCAACAAAGA ATCTATACTT CTTTTTTGTT CTACAAAAAT GCATCCCGAG AGCGCTATTT	1920
TTCTAACAAA GCATCTTAGA TTACTTTTTT TCTCCTTGT GCGCTCTATA ATGCAGTCTC	1980
TTGATAACTT TTTGCACTGT AGGTCCGTTA AGGTTAGAAG AAGGCTACTT TGGTGTCTAT	2040
TTTCTCTTCC ATAAAAAAG CCTGACTCCA CTTCCCGCGT TTACTGATTA CTAGCGAAGC	2100
TGCGGGTGCA TTTTTTCAAG ATAAAGGCAT CCCCATTAT ATTCTATACC GATGTGGATT	2160
GCGCATACTT TGTGAACAGA AAGTGATAGC GTTGATGATT CTTCAATTGGT CAGAAAATTA	2220
TGAACGGTTT CTTCTATTTT GTCTCTATAT ACTACGTATA GGAAATGTTT ACATTTTCGT	2280
ATTGTTTTTCG ATTCACTCTA TGAATAGTTC TTACTACAAT TTTTTTGTCT AAAGAGTAAT	2340
ACTAGAGATA AACATAAAAA ATGTAGAGGT CGAGTTTAGA TGCAAGTTCA AGGAGCGAAA	2400
GGTGGATGGG TAGGTTATAT AGGGATATAG CACAGAGATA TATAGCAAAG AGATACTTTT	2460
GAGCAATGTT TGTGGAAGCG GTATTCGCAA TATTTTAGTA GCTCGTTACA GTCCGGTGCG	2520
TTTTTGTTTT TTTGAAAGTG CGTCTTCAGA GCGCTTTTGG TTTTCAAAG CGCTCTGAAG	2580
TTCCTATACT TTCTAGCTAG AGAATAGGAA CTTCGGAATA GGAACCTCAA AGCGTTTCCG	2640

AAAACGAGCG CTTCCGAAAA TGCAACGCGA GCTGCGCACA TACAGCTCAC TGTTACGTC 2700
GCACCTATAT CTGCGTGTTG CCTGTATATA TATATACATG AGAAGAACGG CATAGTGCGT 2760
GTTTATGCTT AAATGCGTTA TGGTGCACTC TCAGTACAAT CTGCTCTGAT GCCGCATAGT 2820
TAAGCCAGCC CCGACACCCG CCAACACCCG CTGACGCGCC CTGACGGGCT TGTCTGCTCC 2880
CGGCATCCGC TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT 2940
CACCGTCATC ACCGAAACGC GCGAGACGAA AGGGCCTCGT GATACGCCTA TTTTATAGG 3000
TTAATGTCAT GATAATAATG GTTCTTAGA CGTCAGGTGG CACTTTTCGG GGAAATGTGC 3060
GCGGAACCCC TATTTGTTTA TTTTCTAAA TACATTCAA TATGTATCCG CTCATGAGAC 3120
AATAACCCTG ATAAATGCTT CAATAATATT GAAAAAGGAA GAGTATGAGT ATTCAACATT 3180
TCCGTGTCGC CCTTATTCCC TTTTGTGCG CATTGTGCCT TCCTGTTTTT GCTCACCCAG 3240
AAACGCTGGT GAAAGTAAAA GATGCTGAAG ATCAGTTGGG TGCACGAGTG GGTACATCG 3300
AACTGGATCT CAACAGCGGT AAGATCCTTG AGAGTTTTCG CCCGAAGAA CGTTTTCCAA 3360
TGATGAGCAC TTTTAAAGTT CTGCTATGTG GCGCGGTATT ATCCCGTATT GACGCCGGGC 3420
AAGAGCAACT CGGTCGCCGC ATACACTATT CTCAGAATGA CTTGGTTGAG TACTCACCAG 3480
TCACAGAAAA GCATCTTACG GATGGCATGA CAGTAAGAGA ATTATGCAGT GCTGCCATAA 3540
CCATGAGTGA TAACACTGCG GCCAACTTAC TTCTGACAAC GATCGGAGGA CCGAAGGAGC 3600
TAACCGCTTT TTTGCACAAC ATGGGGGATC ATGTAACCTG CCTTGATCGT TGGGAACCGG 3660
AGCTGAATGA AGCCATACCA AACGACGAGC GTGACACCAC GATGCCTGTA GCAATGGCAA 3720
CAACGTTGCG CAACTATTA ACTGGCGAAC TACTTACTCT AGCTTCCCGG CAACAATTAA 3780
TAGACTGGAT GGAGGCGGAT AAAGTTGCAG GACCACTTCT GCGCTCGGCC CTTCCGGCTG 3840
GCTGGTTTAT TGCTGATAAA TCTGGAGCCG GTGAGCGTGG GTCTCGCGGT ATCATTGCAG 3900
CACTGGGGCC AGATGGTAAG CCCTCCCGTA TCGTAGTTAT CTACACGACG GGGAGTCAGG 3960
CAACTATGGA TGAACGAAAT AGACAGATCG CTGAGATAGG TGCCTCACTG ATTAAGCATT 4020
GGTAACTGTC AGACCAAGTT TACTCATATA TACTTTAGAT TGATTTAAAA CTTCATTTTT 4080
AATTTAAAAG GATCTAGGTG AAGATCCTTT TTGATAATCT CATGACCAA ATCCCTTAAC 4140
GTGAGTTTTT GTTCCACTGA GCGTCAGACC CCGTAGAAAA GATCAAAGGA TCTTCTTGAG 4200
ATCCTTTTTT TCTGCGCGTA ATCTGCTGCT TGCAAACAAA AAAACCACCG CTACCAGCGG 4260
TGGTTTGTTT GCCGGATCAA GAGCTACCAA CTCTTTTTC GAAGGTAAC GGCTTCAGCA 4320
GAGCGCAGAT ACCAAATACT GTCCTTCTAG TGTAGCCGTA GTTAGGCCAC CACTTCAAGA 4380
ACTCTGTAGC ACCGCCTACA TACCTCGCTC TGCTAATCCT GTTACCAGTG GCTGCTGCCA 4440
GTGGCGATAA GTCGTGTCTT ACCGGGTTGG ACTCAAGACG ATAGTTACCG GATAAGGCGC 4500

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AGCGGTCGGG CTGAACGGGG GGTTCGTGCA CACAGCCCAG CTTGGAGCGA ACGACCTACA      4560
CCGAACCTGAG ATACCTACAG CGTGAGCATT GAGAAAGCGC CACGCTTCCC GAAGGGAGAA      4620
AGGCGGACAG GTATCCGGTA AGCGGCAGGG TCGGAACAGG AGAGCGCACG AGGGAGCTTC      4680
CAGGGGGAAA CGCCTGGTAT CTTTATAGTC CTGTCGGGTT TCGCCACCTC TGACTTGAGC      4740
GTCGATTTTT GTGATGCTCG TCAGGGGGGC GGAGCCTATG GAAAAACGCC AGCAACGCGG      4800
CCTTTTTTACG GTTCCTGGCC TTTTGCTGGC CTTTGTCTCA CATGTTCTTT CCTGCGTTAT      4860
CCCCTGATTC TGTGGATAAC CGTATTACCG CCTTTGAGTG AGCTGATACC GCTCGCCGCA      4920
GCCGAACGAC CGAGCGCAGC GAGTCAGTGA GCGAGGAAGC GGAAGAGCGC CCAATACGCA      4980
AACCGCCTCT CCCC GCGCGT TGGCCGATTC ATTAATCCAG CTGGCACGAC AGGTTTCCCG      5040
ACTGGAAAGC GGGCAGTGAG CGCAACGCAA TTAATGTGAG TTACCTCACT CATTAGGCAC      5100
CCCAGGCTTT ACACTTTATG CTTCCGGCTC GTATGTTGTG TGAATTGTG AGCGGATAAC      5160
AATTTCACAC AGGAAACAGC T                                          5181

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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 506 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: E. coli methylglyoxal synthase gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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TAAGTGCTTA CAGTAATCTG TAGGAAAGTT AACTACGGAT GTACATTATG GAACTGACGA      60
CTCGCACTTT ACCTGCGCGG AAACATATTG CGCTGGTGGC ACACGATCAC TGCAAACAAA      120
TGCTGATGAG CTGGGTGGAA CGGCATCAAC CGTTACTGGA ACAACACGTA CTGTATGCAA      180
CAGGCACTAC CGGTAACCTA ATTTCCCGCG CGACCGGCAT GAACGTCAAC GCGATGTTGA      240
GTGGCCCAAT GGGGGGTGAC CAGCAGGTTG GCGCATTGAT CTCAGAAGGG AAAATTGATG      300
TATTGATTTT CTTCTGGGAT CCACTAAATG CCGTGCCGCA CGATCCTGAC GTGAAAGCCT      360
TGCTGCGTCT GGCACGGTA TGGAACATTC CGGTCGCCAC CAACGTGGCA ACGGCAGACT      420
TCATAATCCA GTCGCCGCAT TTCAACGACG CGGTCGATAT TCTGATCCCC GATTATCAGC      480
GTTATCTCGC GGACCGTCTG AAGTAA                                          506

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(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3524 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Pyridine nucleotide transhydrogenase gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CATAAAAATA ATCCTTCGCC TTGCGCAAAC CAGGTACTGG TATTGTTATT AACGAGAAAC	60
GTGGCTGATT ATTGCATTTA AACGGTGTAA CTGTCTGCGT CATTTTTCAT ATCACATTCC	120
TTAAGCCAAT TTTAATCCTG CTCAAATGAC CGTCTATGCT TAAAAACAG CCGTATCAGC	180
ATCATTACTA CTGAAGCAAC TGAATTGTAT AAGTTAATTT AATGTTAAGT AGTGATTTCGT	240
GCCGGGGCGA TGTCTCGTTT TACCCGACCG TCGAAGACAA TTATCAGTCT TTATCCGGCG	300
TTCTAAGGTG TTTATCCCAC TATCACGGCT GAATCGTTAA TATTTTGCGA GTTCACGCCG	360
AAATACTGAT TTTTGGCGCT AGATCACAGG CATAATTTTC AGTACGTTAT AGGGCGTTTG	420
TTACTAATTT ATTTTAACGG AGTAACATTT AGCTCGTACA TGAGCAGCTT GTGTGGCTCC	480
TGACACAGGC AAACCATCAT CAATAAAACC GATGGAAGGG AATATCATGC GAATTGGCAT	540
ACCAAGAGAA CGGTTAACCA ATGAAACCCG TGTTCAGCA ACGCCAAAAA CAGTGGAACA	600
GCTGCTGAAA CTGGGTTTTA CCGTCGCGGT AGAGAGCGGC GCGGGTCAAC TGGCAAGTTT	660
TGACGATAAA GCGTTTGTGC AAGCGGGCGC TGAAATTGTA GAAGGGAATA GCGTCTGGCA	720
GTCAGAGATC ATTCTGAAGG TCAATGCGCC GTTAGATGAT GAAATTGCGT TACTGAATCC	780
TGGGACAACG CTGGTGAGTT TTATCTGGCC TCGCAGAAT CCGGAATTAA TGCAAAACT	840
TGCGGAACGT AACGTGACCG TGATGGCGAT GGACTCTGTG CCGCGTATCT CACGCGCACA	900
ATCGCTGGAC GCACTAAGCT CGATGGCGAA CATCGCCGGT TATCGCGCCA TTGTTGAAGC	960
GGCACATGAA TTTGGGCGCT TCTTTACCGG GCAAATTACT GCGGCCGGGA AAGTGCCACC	1020
GGCAAAAGTG ATGGTGATTG GTGCGGGTGT TGCAGGTCTG GCCGCCATTG GCGCAGCAAA	1080
CAGTCTCGGC GCGATTGTGC GTGCATTCTGA CACCCGCCCC GAAGTGAAAG AACAAAGTTCA	1140
AAGTATGGGC GCGGAATTCC TCGAGCTGGA TTTTAAAGAG GAAGCTGGCA GCGGCGATGG	1200
CTATGCCAAA GTGATGTCGG ACGCGTTCAT CAAAGCGGAA ATGGAAGTCT TTGCCGCCCA	1260
GGCAAAAGAG GTCGATATCA TTGTCACCAC CGCGCTTATT CCAGGCAAAC CAGCGCCGAA	1320
GCTAATTACC CGTGAAATGG TTGACTCCAT GAAGGCGGGC AGTGTGATTG TCGACCTGGC	1380

AGCCCAAAAC	GGCGGCAACT	GTGAATACAC	CGTGCCGGGT	GAAATCTTCA	CTACGGAAAA	1440
TGGTGTCAAA	GTGATTGGTT	ATACCGATCT	TCCGGGCCGT	CTGCCGACGC	AATCCTCACA	1500
GCTTTACGGC	ACAAACCTCG	TTAATCTGCT	GAAACTGTTG	TGCAAAGAGA	AAGACGGCAA	1560
TATCACTGTT	GATTTTGATG	ATGTGGTGAT	TCGCGGCGTG	ACCGTGATCC	GTGCGGGCGA	1620
AATTACCTGG	CCGGCACC GC	CGATTCAGGT	ATCAGCTCAG	CCGCAGGCGG	CACAAAAAGC	1680
GGCACCGGAA	GTGAAAACTG	AGGAAAAATG	TACCTGCTCA	CCGTGGCGTA	AATACGCGTT	1740
GATGGCGCTG	GCAATCATTC	TTTTTGGCTG	GATGGCAAGC	GTTGCGCCGA	AAGAATTCCT	1800
TGGGCACTTC	ACCGTTTTCG	CGCTGGCCTG	CGTTGTCGGT	TATTACGTGG	TGTGGAATGT	1860
ATCGCACGCG	CTGCATACAC	CGTTGATGTC	GGTCACCAAC	GCGATTTTCA	GGATTATTGT	1920
TGTCGGAGCA	CTGTTGCAGA	TTGGCCAGGG	CGGCTGGGTT	AGCTTCCTTA	GTTTTATCGC	1980
GGTGCTTATA	GCCAGCATT A	ATATTTTCGG	TGGCTTCACC	GTGACTCAGC	GCATGCTGAA	2040
AATGTTCCGC	AAAAATTAAG	GGGTAACATA	TGTCTGGAGG	ATTAGTTACA	GCTGCATACA	2100
TTGTTGCCGC	GATCCTGTTT	ATCTTCAGTC	TGGCCGGTCT	TTGAAACAT	GAAACGTCTC	2160
GCCAGGGTAA	CAACTTCGGT	ATCGCCGGGA	TGGCGATTGC	GTTAATCGCA	ACCATTTTTG	2220
GACCGGATAC	GGGTAATGTT	GGCTGGATCT	TGCTGGCGAT	GGTCATTGGT	GGGGCAATTG	2280
GTATCCGTCT	GGCGAAGAAA	GTTGAAATGA	CCGAAATGCC	AGAACTGGTG	GCGATCCTGC	2340
ATAGCTTCGT	GGGTCTGGCG	GCAGTGCTGG	TTGGCTTTAA	CAGCTATCTG	CATCATGACG	2400
CGGGAATGGC	ACCGATTCTG	GTCAATATTC	ACCTGACGGA	AGTGTTCTCT	GGTATCTTCA	2460
TCGGGGCGGT	AACGTTACAG	GGTTCGGTGG	TGGCGTTCGG	CAAACGTGTG	GGCAAGATTT	2520
CGTCTAAACC	ATTGATGCTG	CCAAACCGTC	ACAAAATGAA	CCTGGCGGCT	CTGGTCGTTT	2580
CCTTCCTGCT	GCTGATTGTA	TTTGTTTCGA	CGGACAGCGT	CGGCCTGCAA	GTGCTGGCAT	2640
TGCTGATAAT	GACCGCAATT	GCGCTGGTAT	TCGGCTGGCA	TTTAGTCGCC	TCCATCGGTG	2700
GTGCAGATAT	GCCAGTGGTG	GTGTCGATGC	TGAACTCGTA	CTCCGGCTGG	GCGGCTGCGG	2760
CTGCGGGCTT	TATGCTCAGC	AACGACCTGC	TGATTGTGAC	CGGTGCGCTG	GTCGGTTCTT	2820
CGGGGGCTAT	CCTTTCTTAC	ATTATGTGTA	AGGCGATGAA	CCGTTCCTTT	ATCAGCGTTA	2880
TTGCGGGTGG	TTTCGGCACC	GACGGCTCTT	CTACTGGCGA	TGATCAGGAA	GTGGGTGAGC	2940
ACCGCGAAAT	CACCGCAGAA	GAGACAGCGG	AACTGCTGAA	AAACTCCCAT	TCAGTGATCA	3000
TTACTCCGGG	GTACGGCATG	GCAGTCGCGC	AGGCGCAATA	TCCTGTCGCT	GAAATTACTG	3060
AGAAATTGCG	CGCTCGTGGT	ATTAATGTGC	GTTTCGGTAT	CCACCCGGTC	GCGGGGCGTT	3120
TGCCTGGACA	TATGAACGTA	TTGCTGGCTG	AAGCAAAAGT	ACCGTATGAC	ATCGTGCTGG	3180
AAATGGACGA	GATCAATGAT	GACTTTGCTG	ATACCGATAC	CGTACTGGTG	ATTGGTGCTA	3240

ACGATACGGT TAACCCGGCG GCGCAGGATG ATCCGAAGAG TCCGATTGCT GGTATGCCTG 3300
 TGCTGGAAGT GTGGAAAGCG CAGAACGTGA TTGTCTTTAA ACGTTCGATG AACACTGGCT 3360
 ATGCTGGTGT GCAAAACCCG CTGTTCTTCA AGGAAAACAC CCACATGCTG TTTGGTGACG 3420
 CCAAAGCCAG CGTGGATGCA ATCCTGAAAG CTCTGTAACC CTCGACTCTG CTGAGGCCGT 3480
 CACTCTTTAT TGAGATCGCT TAACAGAACG GCGATGCGAC TCTA 3524

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 510 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (v) FRAGMENT TYPE: N-terminal
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Pyridine nucleotide transhydrogenase, subunit A
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
- | | | | | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Arg | Ile | Gly | Ile | Pro | Arg | Glu | Arg | Leu | Thr | Asn | Glu | Thr | Arg | Val | 1 | 5 | 10 | 15 |
| Ala | Ala | Thr | Pro | Lys | Thr | Val | Glu | Gln | Leu | Leu | Lys | Leu | Gly | Phe | Thr | 20 | 25 | 30 | |
| Val | Ala | Val | Glu | Ser | Gly | Ala | Gly | Gln | Leu | Ala | Ser | Phe | Asp | Asp | Lys | 35 | 40 | 45 | |
| Ala | Phe | Val | Gln | Ala | Gly | Ala | Glu | Ile | Val | Glu | Gly | Asn | Ser | Val | Trp | 50 | 55 | 60 | |
| Gln | Ser | Glu | Ile | Ile | Leu | Lys | Val | Asn | Ala | Pro | Leu | Asp | Asp | Glu | Ile | 65 | 70 | 75 | 80 |
| Ala | Leu | Leu | Asn | Pro | Gly | Thr | Thr | Leu | Val | Ser | Phe | Ile | Trp | Pro | Ala | 85 | 90 | 95 | |
| Gln | Asn | Pro | Glu | Leu | Met | Gln | Lys | Leu | Ala | Glu | Arg | Asn | Val | Thr | Val | 100 | 105 | 110 | |
| Met | Ala | Met | Asp | Ser | Val | Pro | Arg | Ile | Ser | Arg | Ala | Gln | Ser | Leu | Asp | 115 | 120 | 125 | |
| Ala | Leu | Ser | Ser | Met | Ala | Asn | Ile | Ala | Gly | Tyr | Arg | Ala | Ile | Val | Glu | 130 | 135 | 140 | |
| Ala | Ala | His | Glu | Phe | Gly | Arg | Phe | Phe | Thr | Gly | Gln | Ile | Thr | Ala | Ala | 145 | 150 | 155 | 160 |
| Gly | Lys | Val | Pro | Pro | Ala | Lys | Val | Met | Val | Ile | Gly | Ala | Gly | Val | Ala | | | | |

165								170				175			
Gly	Leu	Ala	Ala	Ile	Gly	Ala	Ala	Asn	Ser	Leu	Gly	Ala	Ile	Val	Arg
			180					185					190		
Ala	Phe	Asp	Thr	Arg	Pro	Glu	Val	Lys	Glu	Gln	Val	Gln	Ser	Met	Gly
		195					200					205			
Ala	Glu	Phe	Leu	Glu	Leu	Asp	Phe	Lys	Glu	Glu	Ala	Gly	Ser	Gly	Asp
	210					215					220				
Gly	Tyr	Ala	Lys	Val	Met	Ser	Asp	Ala	Phe	Ile	Lys	Ala	Glu	Met	Glu
225					230					235					240
Leu	Phe	Ala	Ala	Gln	Ala	Lys	Glu	Val	Asp	Ile	Ile	Val	Thr	Thr	Ala
				245					250					255	
Leu	Ile	Pro	Gly	Lys	Pro	Ala	Pro	Lys	Leu	Ile	Thr	Arg	Glu	Met	Val
			260					265					270		
Asp	Ser	Met	Lys	Ala	Gly	Ser	Val	Ile	Val	Asp	Leu	Ala	Ala	Gln	Asn
		275					280					285			
Gly	Gly	Asn	Cys	Glu	Tyr	Thr	Val	Pro	Gly	Glu	Ile	Phe	Thr	Thr	Glu
	290					295					300				
Asn	Gly	Val	Lys	Val	Ile	Gly	Tyr	Thr	Asp	Leu	Pro	Gly	Arg	Leu	Pro
305					310					315					320
Thr	Gln	Ser	Ser	Gln	Leu	Tyr	Gly	Thr	Asn	Leu	Val	Asn	Leu	Leu	Lys
				325					330					335	
Leu	Leu	Cys	Lys	Glu	Lys	Asp	Gly	Asn	Ile	Thr	Val	Asp	Phe	Asp	Asp
			340					345					350		
Val	Val	Ile	Arg	Gly	Val	Thr	Val	Ile	Arg	Ala	Gly	Glu	Ile	Thr	Trp
		355					360					365			
Pro	Ala	Pro	Pro	Ile	Gln	Val	Ser	Ala	Gln	Pro	Gln	Ala	Ala	Gln	Lys
	370					375					380				
Ala	Ala	Pro	Glu	Val	Lys	Thr	Glu	Glu	Lys	Cys	Thr	Cys	Ser	Pro	Trp
385					390					395					400
Arg	Lys	Tyr	Ala	Leu	Met	Ala	Leu	Ala	Ile	Ile	Leu	Phe	Gly	Trp	Met
				405					410					415	
Ala	Ser	Val	Ala	Pro	Lys	Glu	Phe	Leu	Gly	His	Phe	Thr	Val	Phe	Ala
			420					425					430		
Leu	Ala	Cys	Val	Val	Gly	Tyr	Tyr	Val	Val	Trp	Asn	Val	Ser	His	Ala
		435					440					445			
Leu	His	Thr	Pro	Leu	Met	Ser	Val	Thr	Asn	Ala	Ile	Ser	Gly	Ile	Ile
	450					455					460				
Val	Val	Gly	Ala	Leu	Leu	Gln	Ile	Gly	Gln	Gly	Gly	Trp	Val	Ser	Phe
465					470					475					480
Leu	Ser	Phe	Ile	Ala	Val	Leu	Ile	Ala	Ser	Ile	Asn	Ile	Phe	Gly	Gly
				485					490					495	

Phe Thr Val Thr Gln Arg Met Leu Lys Met Phe Arg Lys Asn
 500 505 510

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 462 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Pyridine nucleotide transhydrogenase, subunit B

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Ser Gly Gly Leu Val Thr Ala Ala Tyr Ile Val Ala Ala Ile Leu
 1 5 10 15

Phe Ile Phe Ser Leu Ala Gly Leu Ser Lys His Glu Thr Ser Arg Gln
 20 25 30

Gly Asn Asn Phe Gly Ile Ala Gly Met Ala Ile Ala Leu Ile Ala Thr
 35 40 45

Ile Phe Gly Pro Asp Thr Gly Asn Val Gly Trp Ile Leu Leu Ala Met
 50 55 60

Val Ile Gly Gly Ala Ile Gly Ile Arg Leu Ala Lys Lys Val Glu Met
 65 70 75 80

Thr Glu Met Pro Glu Leu Val Ala Ile Leu His Ser Phe Val Gly Leu
 85 90 95

Ala Ala Val Leu Val Gly Phe Asn Ser Tyr Leu His His Asp Ala Gly
 100 105 110

Met Ala Pro Ile Leu Val Asn Ile His Leu Thr Glu Val Phe Leu Gly
 115 120 125

Ile Phe Ile Gly Ala Val Thr Phe Thr Gly Ser Val Val Ala Phe Gly
 130 135 140

Lys Leu Cys Gly Lys Ile Ser Ser Lys Pro Leu Met Leu Pro Asn Arg
 145 150 155 160

His Lys Met Asn Leu Ala Ala Leu Val Val Ser Phe Leu Leu Leu Ile
 165 170 175

Val Phe Val Arg Thr Asp Ser Val Gly Leu Gln Val Leu Ala Leu Leu
 180 185 190

Ile Met Thr Ala Ile Ala Leu Val Phe Gly Trp His Leu Val Ala Ser
 195 200 205

Ile	Gly	Gly	Ala	Asp	Met	Pro	Val	Val	Val	Ser	Met	Leu	Asn	Ser	Tyr
210						215					220				
Ser	Gly	Trp	Ala	Ala	Ala	Ala	Ala	Gly	Phe	Met	Leu	Ser	Asn	Asp	Leu
225					230					235					240
Leu	Ile	Val	Thr	Gly	Ala	Leu	Val	Gly	Ser	Ser	Gly	Ala	Ile	Leu	Ser
				245					250					255	
Tyr	Ile	Met	Cys	Lys	Ala	Met	Asn	Arg	Ser	Phe	Ile	Ser	Val	Ile	Ala
			260					265					270		
Gly	Gly	Phe	Gly	Thr	Asp	Gly	Ser	Ser	Thr	Gly	Asp	Asp	Gln	Glu	Val
		275					280					285			
Gly	Glu	His	Arg	Glu	Ile	Thr	Ala	Glu	Glu	Thr	Ala	Glu	Leu	Leu	Lys
	290					295					300				
Asn	Ser	His	Ser	Val	Ile	Ile	Thr	Pro	Gly	Tyr	Gly	Met	Ala	Val	Ala
305					310					315					320
Gln	Ala	Gln	Tyr	Pro	Val	Ala	Glu	Ile	Thr	Glu	Lys	Leu	Arg	Ala	Arg
				325					330					335	
Gly	Ile	Asn	Val	Arg	Phe	Gly	Ile	His	Pro	Val	Ala	Gly	Arg	Leu	Pro
			340					345					350		
Gly	His	Met	Asn	Val	Leu	Leu	Ala	Glu	Ala	Lys	Val	Pro	Tyr	Asp	Ile
		355					360					365			
Val	Leu	Glu	Met	Asp	Glu	Ile	Asn	Asp	Asp	Phe	Ala	Asp	Thr	Asp	Thr
	370					375					380				
Val	Leu	Val	Ile	Gly	Ala	Asn	Asp	Thr	Val	Asn	Pro	Ala	Ala	Gln	Asp
385					390					395					400
Asp	Pro	Lys	Ser	Pro	Ile	Ala	Gly	Met	Pro	Val	Leu	Glu	Val	Trp	Lys
				405					410					415	
Ala	Gln	Asn	Val	Ile	Val	Phe	Lys	Arg	Ser	Met	Asn	Thr	Gly	Tyr	Ala
			420					425					430		
Gly	Val	Gln	Asn	Pro	Leu	Phe	Phe	Lys	Glu	Asn	Thr	His	Met	Leu	Phe
		435					440					445			
Gly	Asp	Ala	Lys	Ala	Ser	Val	Asp	Ala	Ile	Leu	Lys	Ala	Leu		
	450					455					460				

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1139 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Glycerol dehydrogenase gene
(B) STRAIN: E. coli

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```

TACGGCGTAA ACCGTGATGA GTAGAGATTT CCTCGTTAAT ACCTGGCGTA ATAAGTTAGT      60
GGCCCATTTA TGTAGGTCCC GCGACTACAC TAATTAGCAG ACCCGCTTAT GGACTTCGGC      120
GACTCTTGCG ACCAATCACC ACCCACTGTT TAAACAAAAT CCAAACGAG TTAGGTGACA      180
GCTCTTTTTCG AAATTTCTAC GACCAGACCA TCATCTTTAA CGCGGCAAAC CGCCACTTAC      240
AAGCGTTTTTA CTCATGCTGG CAGACGCACC GTAGCGCCTC TGACGCGTCA CACCGCGTTA      300
AGAGCCATAG CCACCGCCTT TTTGGGAGCT ATGACGGTTT CGTGACCGTG TAAAGTACCC      360
ACAAGGCCAT CGCTAGCGTG GCTGATAGCG GAGATGGCTA CGTGGCACGT CGCGTAACAG      420
ACAATAGATG TGGCTACTCC CACTCAAAC TGGCGATAGAC GACAACGGTT TATTGGGCTT      480
ATACCAGTAA CAGCTGTGGT TTTAGCAGCG ACCGCGTGGA CGTGCAGACA ATCGCCGCCC      540
ATAGCCGCTA CGCGACCGTT GGACCAAAC TCGCGCACGG ACGAGAGCAT CGCCGCGCTG      600
GTGGTACCGC CCGCCGTTCA CGTGGGTCCG ACGCGACCGT GACCGACTTG ACACGATGTT      660
GTGGGACGAC CTTCTTCCGC TTTTTCGCTA CGAACGACGG CTTGTCATGC ATCACTGAGG      720
CCGCGACCTC GCGCACTAAC TTCGCTTG TG GATAAACTCG CCACAACCAA AACTTTCACC      780
ACCAGACGAC GCCGCGTGCG TCACGTATTG CCGGACTGGC GATAGGGCCT GCGCGTAGTG      840
ATAATAGTGC CACTTTTTCA CCGTAAGCCA TGCGACTGCG TCGACCAAGA CCTTTTACGC      900
GGCCACCTCC TTTAGCTTTG GCATCGACGG GAATCGGTAC GCCATCCAAA CGTTATTGAG      960
AGCGAGTTGA CCTATAATTT CTTCTACAGG GCCCGTTTTA CGCTTAACAC CGTCTTCGCC     1020
GTACACGTCT TCCACTTTGG TAAGTGTTGT ACGGACCGCC GCGCTGCGGT CTAGTCCAAA     1080
TGCGGCGAGA CGACCATCGG CTGGTCATGC CAGTCGCAAA GGACGTTCTC ACCCTTATT     1139

```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 380 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: E. coli glycerol dehydrogenase

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met	Pro	His	Leu	Ala	Leu	Leu	Ile	Ser	Lys	Gly	Ala	Ile	Met	Asp	Arg			
1				5					10					15				
Ile	Ile	Gln	Ser	Pro	Gly	Lys	Tyr	Ile	Gln	Gly	Ala	Asp	Val	Ile	Asn			
			20					25					30					
Arg	Leu	Gly	Glu	Tyr	Leu	Lys	Pro	Leu	Xaa	Glu	Arg	Trp	Leu	Val	Val			
		35					40					45						
Gly	Asp	Lys	Phe	Val	Leu	Gly	Phe	Ala	Gln	Ser	Thr	Val	Glu	Lys	Ser			
	50					55					60							
Phe	Lys	Asp	Ala	Gly	Leu	Val	Val	Glu	Ile	Ala	Pro	Phe	Gly	Gly	Glu			
65					70					75					80			
Cys	Ser	Gln	Asn	Glu	Ile	Asp	Arg	Leu	Arg	Gly	Ile	Ala	Glu	Thr	Ala			
				85					90					95				
Gln	Cys	Gly	Ala	Ile	Leu	Gly	Ile	Gly	Gly	Gly	Lys	Thr	Leu	Asp	Thr			
			100					105					110					
Ala	Lys	Ala	Leu	Ala	His	Phe	Met	Gly	Val	Pro	Val	Ala	Ile	Ala	Pro			
		115					120					125						
Thr	Ile	Ala	Ser	Thr	Asp	Ala	Pro	Cys	Ser	Ala	Leu	Ser	Val	Ile	Tyr			
	130					135					140							
Thr	Asp	Glu	Gly	Glu	Phe	Asp	Arg	Tyr	Leu	Leu	Leu	Pro	Asn	Asn	Pro			
145					150					155					160			
Asn	Met	Val	Ile	Val	Asp	Thr	Lys	Ile	Val	Ala	Gly	Ala	Pro	Ala	Arg			
				165					170					175				
Leu	Leu	Ala	Ala	Gly	Ile	Gly	Asp	Ala	Leu	Ala	Thr	Trp	Phe	Glu	Ala			
			180					185					190					
Arg	Ala	Cys	Ser	Arg	Ser	Gly	Ala	Thr	Thr	Met	Ala	Gly	Gly	Lys	Cys			
		195					200					205						
Thr	Gln	Ala	Ala	Leu	Ala	Leu	Ala	Glu	Leu	Cys	Tyr	Asn	Thr	Leu	Leu			
	210					215					220							
Glu	Glu	Gly	Glu	Lys	Ala	Met	Leu	Ala	Ala	Glu	Gln	His	Val	Val	Thr			
225					230					235					240			
Pro	Ala	Leu	Glu	Arg	Val	Ile	Glu	Ala	Asn	Thr	Tyr	Leu	Ser	Gly	Val			
				245					250					255				
Gly	Phe	Glu	Ser	Gly	Gly	Leu	Ala	Ala	Ala	His	Ala	Val	His	Asn	Gly			
			260					265					270					
Leu	Thr	Ala	Ile	Pro	Asp	Ala	His	His	Tyr	Tyr	His	Gly	Glu	Lys	Val			
		275					280					285						
Ala	Phe	Gly	Thr	Leu	Thr	Gln	Leu	Val	Leu	Glu	Asn	Ala	Pro	Val	Glu			
	290					295					300							
Glu	Ile	Glu	Thr	Val	Ala	Ala	Leu	Ser	His	Ala	Val	Gly	Leu	Pro	Ile			
305					310					315					320			
Thr	Leu	Ala	Gln	Leu	Asp	Ile	Lys	Glu	Asp	Val	Pro	Ala	Lys	Met	Arg			
				325					330					335				

Ile Val Ala Glu Ala Ala Cys Ala Glu Gly Glu Thr Ile His Asn Met
340 345 350
Pro Gly Gly Ala Thr Pro Asp Gln Val Tyr Ala Ala Leu Leu Val Ala
355 360 365
Asp Gln Tyr Gly Gln Arg Phe Leu Gln Glu Trp Glu
370 375 380

BIBLIOGRAPHY

- Ackerman, R.S., Cozzarelli, R., Epstein, W. (1974) *J. Bact.* 119:357-362.
- Cameron, D.C. and C.L. Cooney (1986) *Bio/Technology* 4:651-654.
- Ferguson, G.P., Chacko, A.D., Lee, C., Booth I.R. (1996) *J. Bact.* 178:3957-3961.
- Franklin Associates, Ltd. (1994) "Life Cycle Assessment of Ethylene Glycol and Propylene Glycol Based Antifreeze," Franklin Associates, Prairie Village, Kansas.
- Freedberg, W.B., Kistler, W.S., Lin, E.C.C. (1971) *J. Bact.* 108:137-144.
- Gait, A.J. "Propylene Oxide," In: E.G. Hancock (ed.), *Propylene and its Industrial Derivatives*, p. 273-297, John Wiley and Sons, NY, NY (1973).
- Ghalambor, M.A.; Heath, E.C. (1962) *J. Biol. Chem.* 237:2427-2433.
- Hill, J.E., Myers, A.M., Koerner, T.J., Tzagoloff, A. (1986) *Yeast* 2:163-167.
- Hopper, D.J., and Cooper, R.A. (1972) *Biochem J.* 128:321-329.
- Kadner, R.J., Murphy, G.P., Stephens, C.M. (1992) *J. Gen. Microbio.* 138:2007-2014.
- Kluyver and Schnellen (1937) *Enzymologia* 4:7-12.
- Koob, M.D., Shaw, A.J., and Cameron, D.C. (1994) *Proc. N.Y. Acad. Sci.* 745:1-3.
- Obradors, N., Badia, J., Baldoma, L., and Aguilar, J. (1988) *J. Bact.* 170:2159-2162.
- Old, S.E., Sato, S., Kador, P.F., and Carper, D.A. (1990) *Proc. Natl. Acad. Sci.* 87:4942-4945.
- Percy, D.S. and Harrison, D.H.T. (1996) Cloning, Expression and Characterization of Methylglyoxal Synthase from *Escherichia coli*. *American Society for Biochemistry and Molecular Biology Meeting Abstract, Protein Structure Section*, No. 1367, June 2-6, 1996.
- Sambrook, Fritsch, and Maniatis (1986) "Molecular Cloning, A Laboratory Manual," 2nd. Ed.
- Sato, S., Old, S., Carper, D., and Kador, P.F. (1995) *Enzymology and Molecular Biology of Carbonyl Metabolism 5*, p. 259-268, H. Weiner et al. (Eds.), Plenum Press, NY.

Sawada, H.; Takagi, Y. (1964) *Biochim. Biophys. Acta*, 92:26-32.

Simon, E.S., Whitesides, G.M., Cameron, D.C., Weitz, D.J., and Cooney, C.L.
(1987) *J. Org. Chem.* 52:4042-4044.

Sridhara, S.; Wu, T.T. (1969) *J. Biol. Chem.* 244:5233-5238.

Tran-Din, K. and G. Gottschalk (1985) *Arch. Microbiol.* 142:87-92.

CLAIMS

What is claimed is:

1. A method of producing 1,2-propanediol by fermentation of sugars comprising: culturing a recombinant microorganism which expresses one or more enzymes which catalyze production of 1,2-propanediol from intracellular methylglyoxal in a medium containing a sugar carbon source other than a 6-deoxyhexose sugar, whereby the sugar carbon source is metabolized by the microorganism into 1,2-propanediol.
2. The method of Claim 1, wherein a recombinant microorganism containing one or more recombinant genes whose encoded gene products catalyze the reduction of methylglyoxal to 1,2-propanediol is cultured.
3. The method of Claim 1 or Claim 2, wherein a recombinant *E. coli* is cultured.
4. The method of any one of Claims 1,2, or 3, wherein a recombinant microorganism which expresses enzyme activity selected from the group consisting of recombinant aldose reductase activity, recombinant glycerol dehydrogenase activity, recombinant methylglyoxal synthase activity, recombinant pyridine nucleotide transferase, and combinations thereof, is cultured.
5. The method according to any one of the preceding claims, wherein a recombinant microorganism transformed with a transformation vector containing a gene sequence selected from the group consisting of SEQ. ID. NO: 3, SEQ. ID. NO: 6, SEQ. ID. NO: 7, SEQ. ID. NO: 10, and combinations thereof, is cultured.
6. The method according to any one of the preceding claims, wherein a recombinant microorganism transformed with a transformation vector containing a gene sequence selected from the group consisting of SEQ. ID. NO: 3, SEQ. ID. NO: 6, SEQ. ID. NO: 7, SEQ. ID. NO: 10, and combinations thereof, the gene sequence operationally linked to one or more promoter sequences whereby transcription of the gene sequence is controlled, is cultured.

7. The method of Claim 6, wherein the promoter sequence is selected from the group consisting of *lac*, *trc*, *tac*, and *phoA*.

8. The method according to any one of the preceding claims, wherein a microorganism lacking enzyme activity selected from the group consisting of triose phosphate isomerase activity, glyoxalase I activity, and combinations thereof, is cultured.

9. The method according to any one of the preceding claims, wherein the microorganism is cultured in a medium containing a sugar carbon source selected from the group consisting of arabinose, fructose, galactose, glucose, lactose, maltose, sucrose, xylose, and combinations thereof.

10. The method according to any one of the preceding claims, wherein the microorganism is cultured aerobically.

11. The method according to any one of the preceding claims, wherein the microorganism is cultured anaerobically.

12. The method according to any one of the preceding claims, further comprising the step of isolating the 1,2-propanediol formed.

13. The method according to any one of the preceding claims, wherein the microorganism is cultured under conditions favorable to the production of intracellular methylglyoxal.

14. The method according to any one of the preceding claims, wherein a recombinant microorganism further containing a recombinant methylglyoxal synthase gene is cultured.

15. The method according to any one of the preceding claims, wherein a recombinant microorganism further containing a recombinant pyridine nucleotide transferase gene is cultured.

16. A synthetic operon which enables the production of 1,2-propanediol in a microorganism transformed to contain the operon, the operon comprising one or more genes whose encoded gene products catalyze the reduction

of methylglyoxal to 1,2-PD and a promoter sequence operationally linked to the one or more genes.

17. The synthetic operon of Claim 16, wherein the one or more genes are selected from the group consisting of an aldose reductase gene, a glycerol dehydrogenase gene, and combinations thereof.

18. The synthetic operon of Claim 16 or 17, further comprising one or more genes whose encoded gene products catalyze increased production of intracellular methylglyoxal.

19. The synthetic operon of any one of Claims 16, 17, or 18, comprising a methylglyoxal synthase gene.

20. A synthetic operon comprising at least one promoter sequence, a gene selected from the group consisting of an aldose reductase gene, a glycerol dehydrogenase gene, and combinations thereof; and a gene selected from the group consisting of a methylglyoxal synthase gene, a pyridine nucleotide transferase gene, and combinations thereof, wherein the genes are operationally linked to the at least one promoter.

21. The synthetic operon of Claim 20, comprising SEQ. ID. NO: 3, SEQ. ID. NO: 6, and SEQ. ID. NO: 7.

22. The synthetic operon of Claim 20 or 21, comprising SEQ. ID. NO: 10 and SEQ. ID. NO: 6.

23. An *E. coli* transformed with a synthetic operon as recited in any one of Claims 16-22.

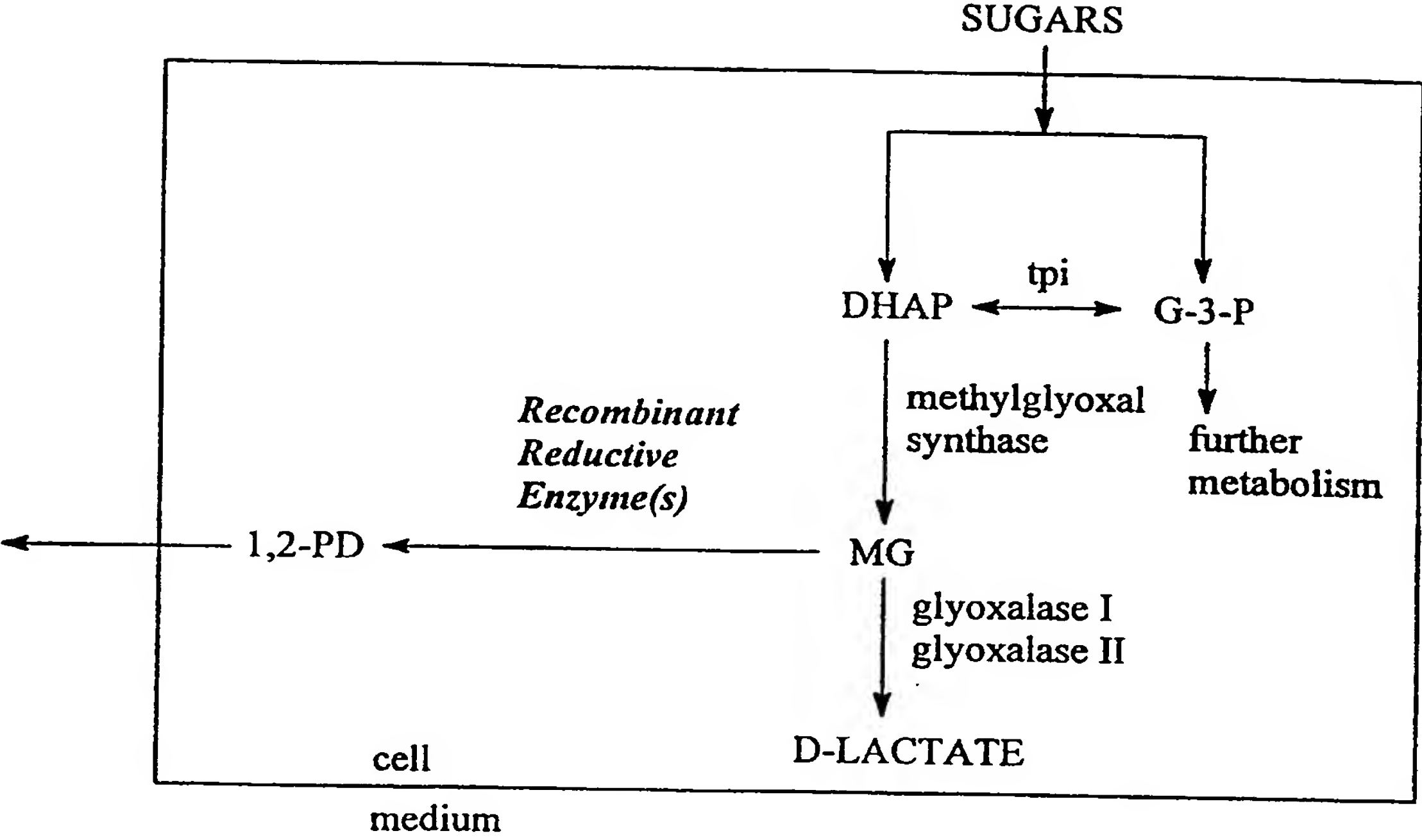


FIG. 1

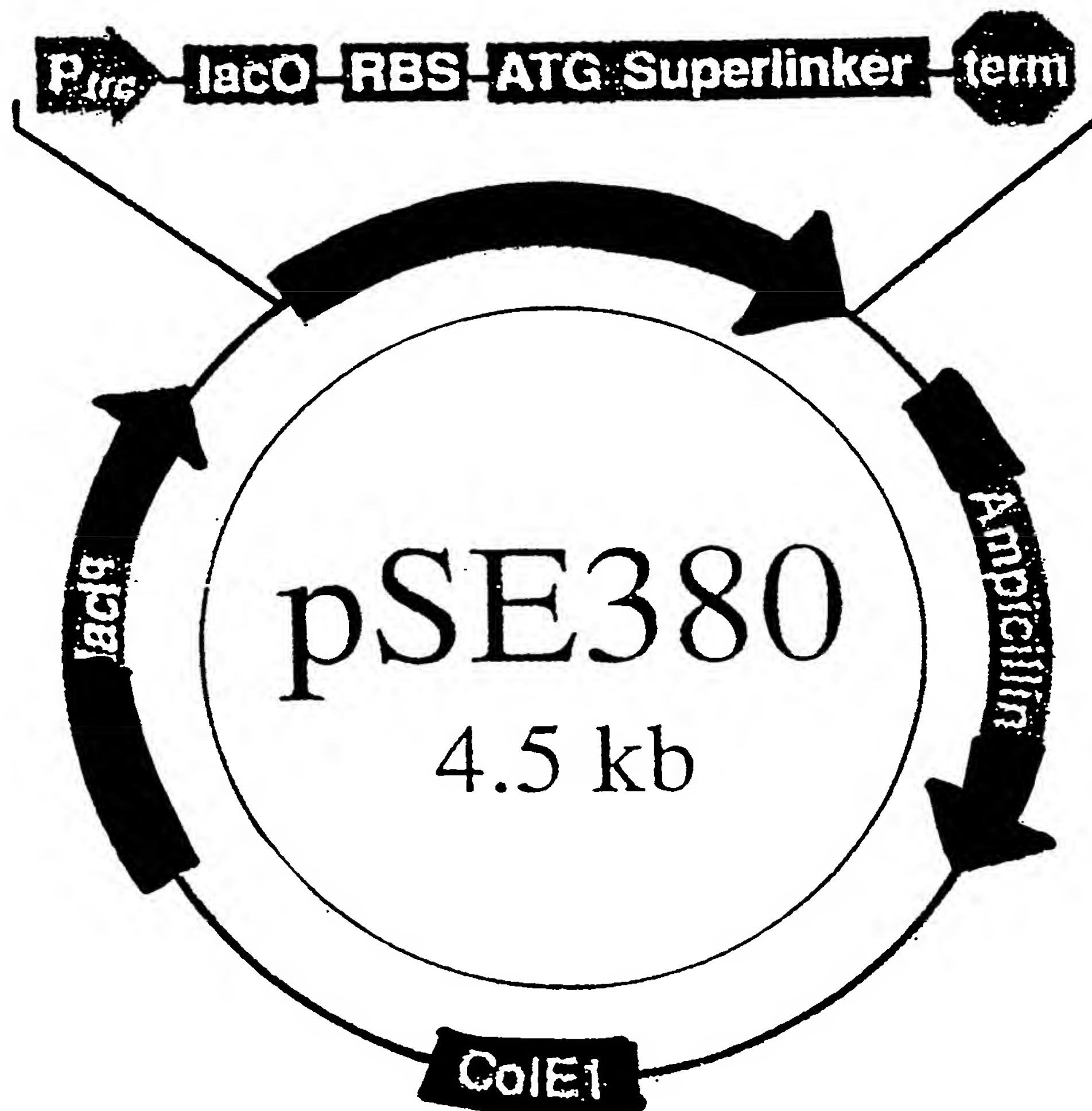


FIG. 2

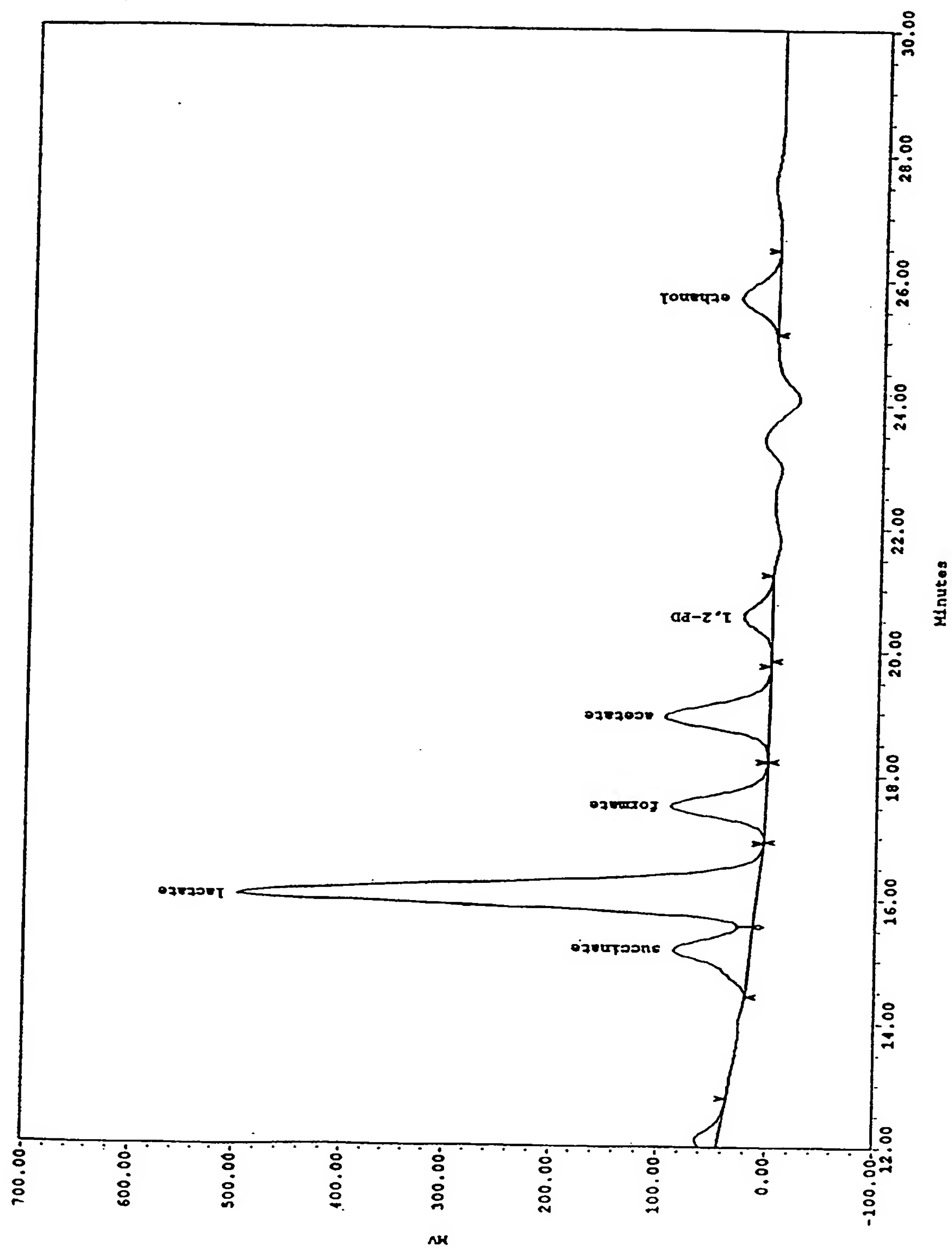


FIG. 3

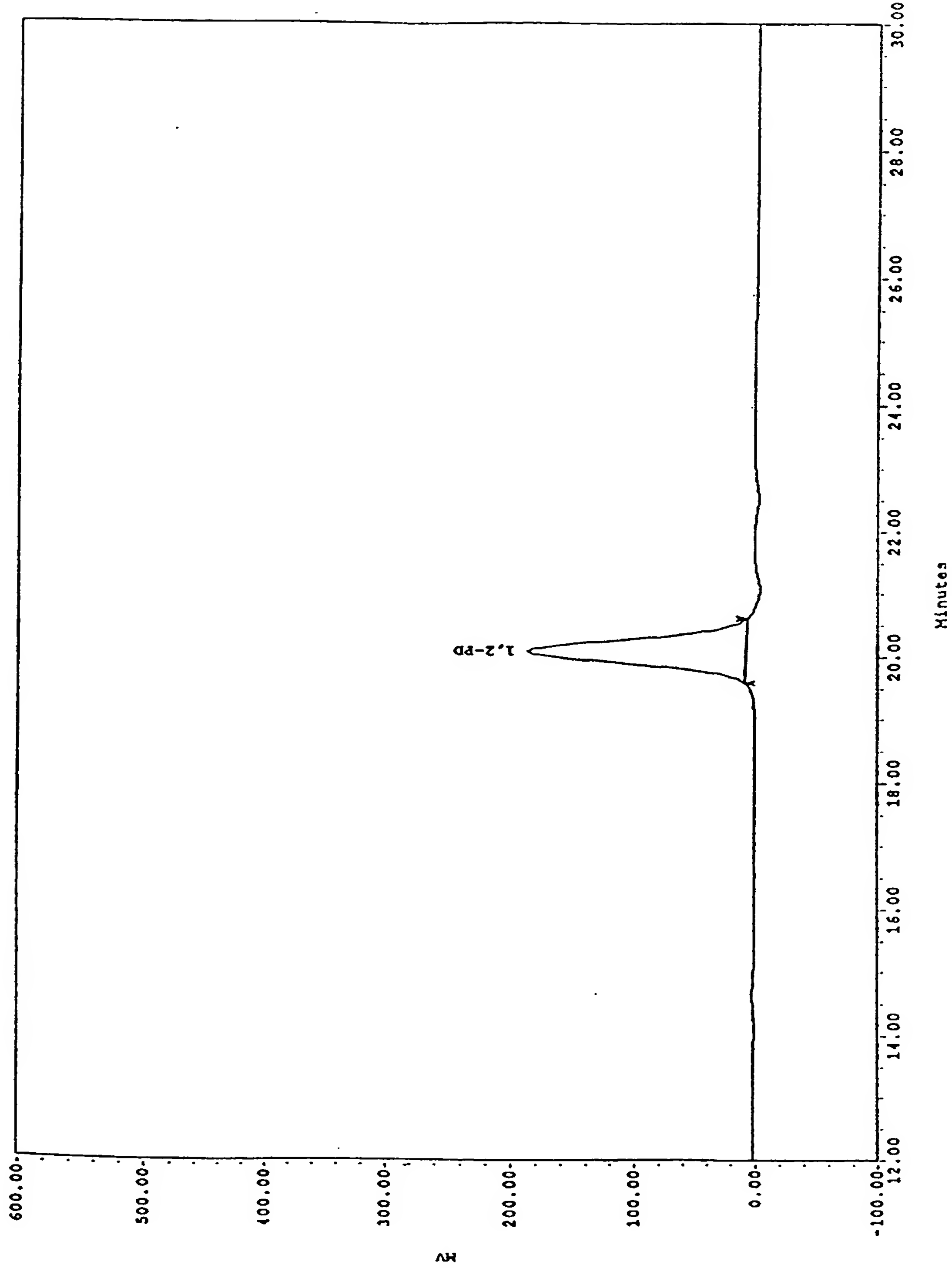


FIG. 4

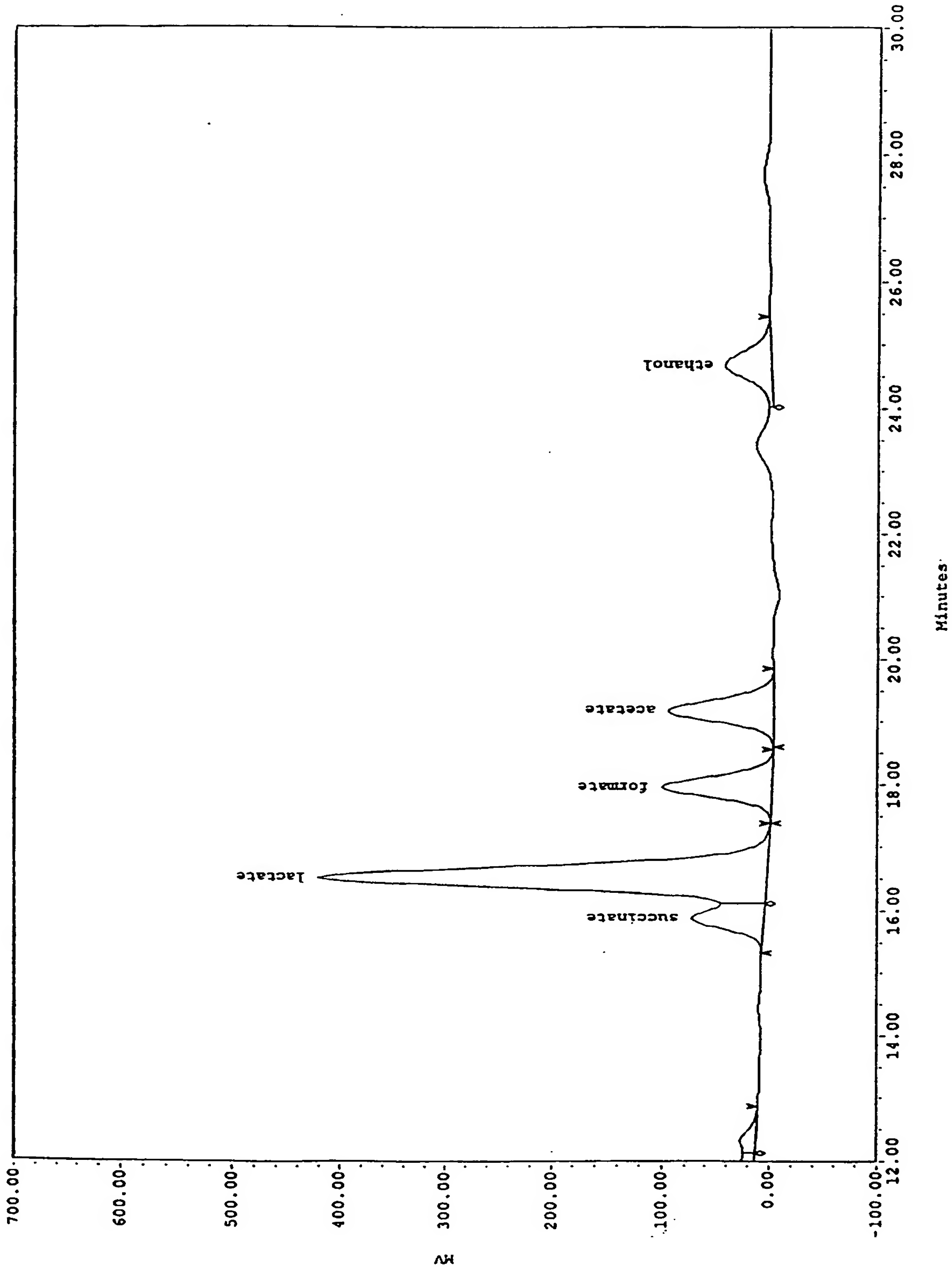


FIG. 5

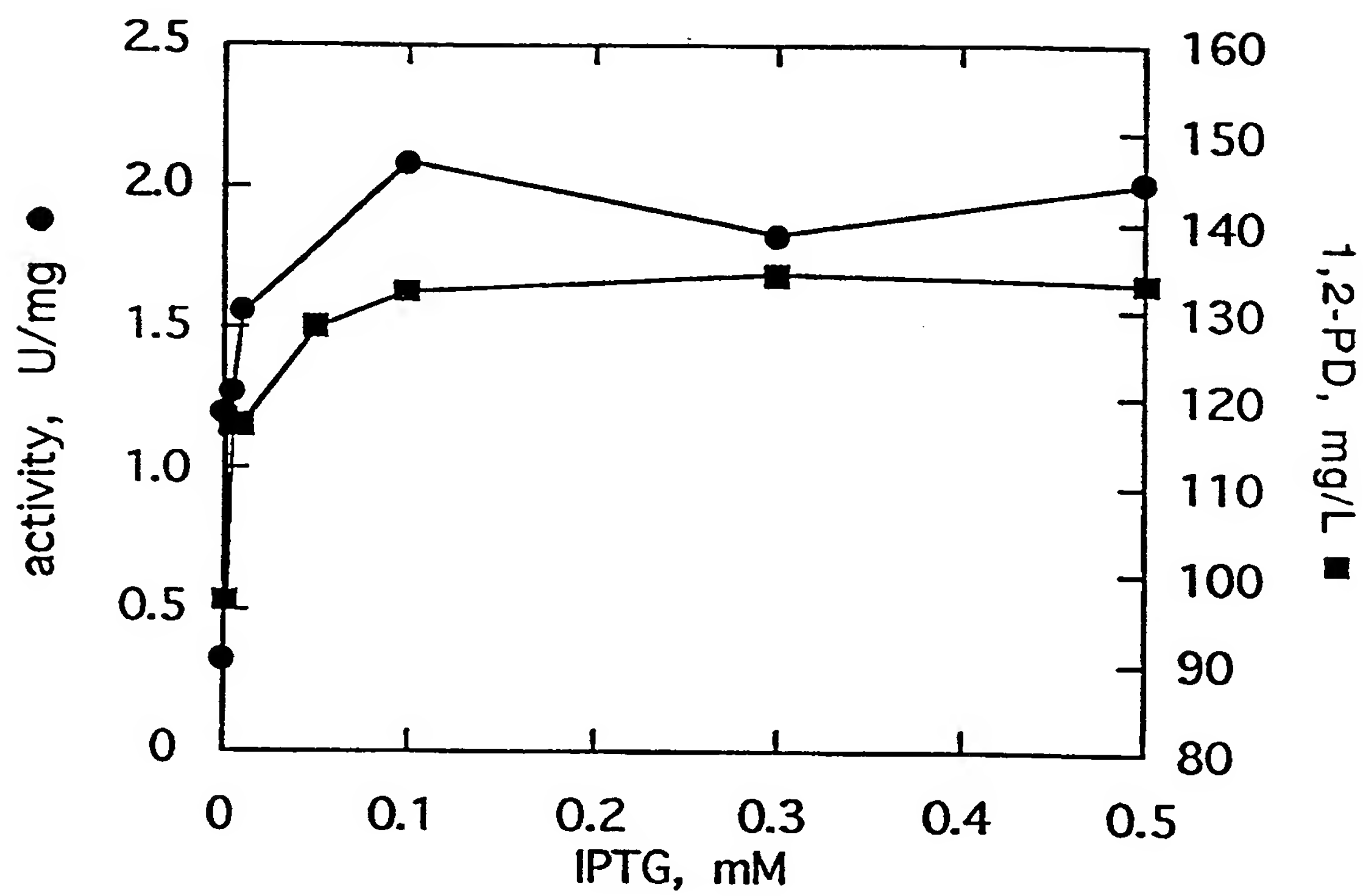


FIG. 6

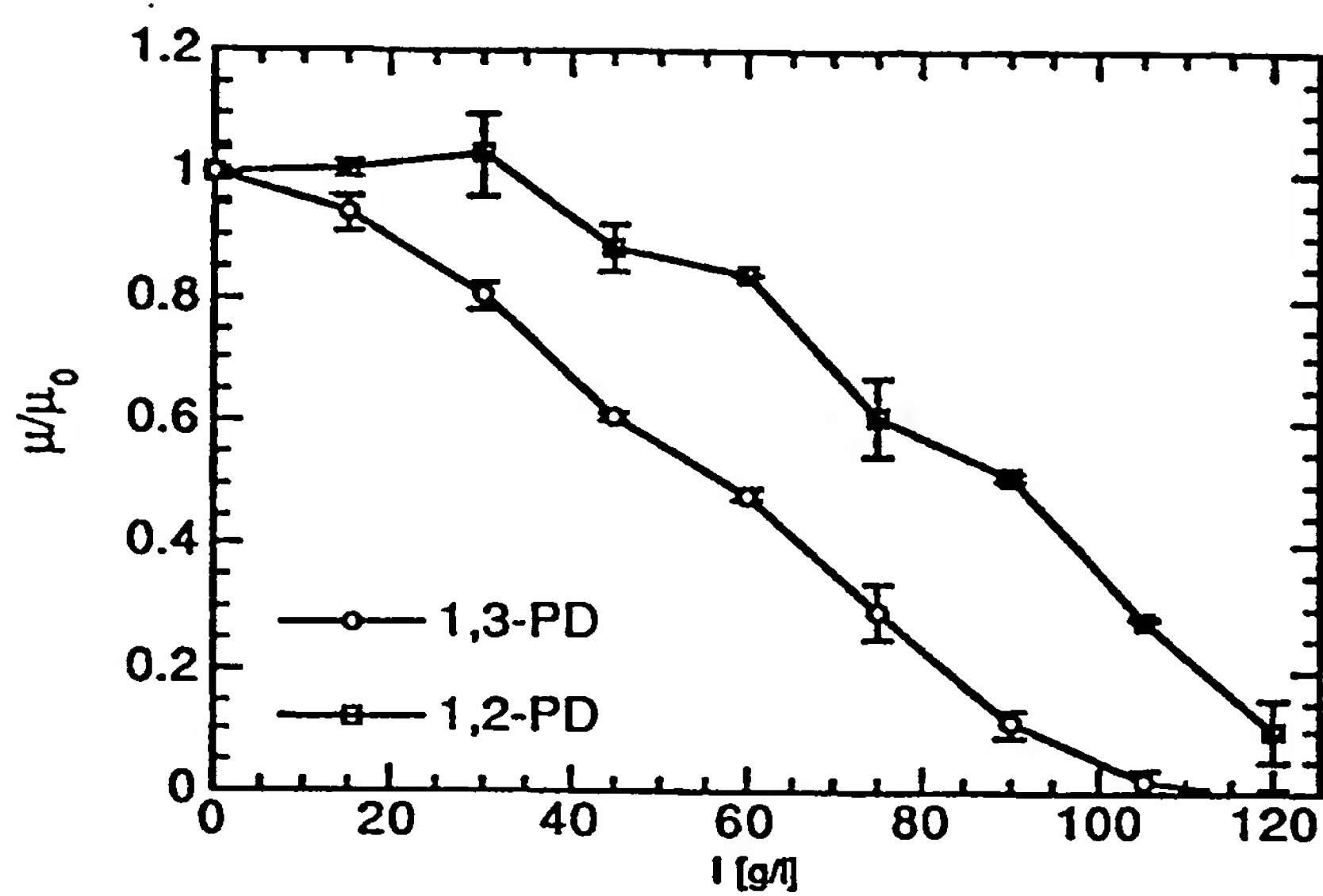


FIG. 7

INTERNATIONAL SEARCH REPORT

national Application No
PCT/US 98/03271

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/53 C12P7/18
//(C12P7/18,C12R1:19)

C12N9/04

C12N1/21

C12N9/88

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	CAMERON D.C. ET AL.: "Metabolic engineering of propanediol pathways." BIOTECHNOLOGY PROGRESS, vol. 14, no. 1, 6 February 1998, pages 116-125, XP002067772 see abstract see figure 1 see page 120, column 2 - page 121, column 1 --- -/--	1-23

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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- "&" document member of the same patent family

Date of the actual completion of the international search

17 June 1998

Date of mailing of the international search report

30/06/1998

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Authorized officer

Lejeune, R

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/03271

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CAMERON D.C. & COONEY C.L.: "A novel fermentation: the production of R(-)-1,2-propanediol and acetol by <i>Clostridium thermosaccharolyticum</i> ." BIO/TECHNOLOGY, vol. 4, 1986, XP002067773 cited in the application see abstract see figure 3	1,9-12
X	OLD S.E. ET AL.: "In vitro expression of rat lens aldose reductase in <i>Escherichia coli</i> ." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 87, July 1990, pages 4942-4945, XP002067774 cited in the application see abstract	16,17,23
X	TRUNIGER V. & BOOS W.: "Mapping and cloning of <i>gldA</i> , the structural gene of the <i>Escherichia coli</i> glycerol dehydrogenase." JOURNAL OF BACTERIOLOGY, vol. 176, no. 6, March 1994, pages 1796-1800, XP002067775 see abstract	16,17,23
X	HILL P.W. ET AL.: "Bioenergetics and end-product regulation of <i>Clostridium thermosaccharolyticum</i> in response to nutrient limitation." BIOTECHNOLOGY AND BIOENGINEERING, vol. 43, 1993, pages 873-883, XP002067776 see figure 1 see figures 6-9	1,9-12
Y	TRAN-DIN K. & GOTTSCHALK G.: "Formation of D(-)-1,2-propanediol and D(-)-lactate from glucose by <i>Clostridium sphenoides</i> under phosphate limitation." ARCHIVES OF MICROBIOLOGY, vol. 142, 1985, pages 87-92, XP002067777 cited in the application see abstract see figure 2 see page 90, column 2, paragraph 4 - page 91, column 1, paragraph 2	1-7,9-13
Y		2-7,13

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INTERNATIONAL SEARCH REPORT

national Application No

PCT/US 98/03271

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	VANDER JAGT D.L. ET AL.: "Reduction of trioses by NADPH-dependent aldo-keto reductases." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 267, no. 7, 5 March 1992, pages 4364-4369, XP002067778 see abstract see page 4367, column 2, scheme 1 -----	1-7,9-13
Y	LEE L.G. & WHITESIDES G.M.: "Preparation of optically active 1,2-diols and alpha-hydroxy ketones using glycerol dehydrogenase as catalyst: limits to enzyme-catalyzed synthesis due to noncompetitive and mixed inhibition by product." JOURNAL OF ORGANIC CHEMISTRY, vol. 51, no. 1, 1986, pages 25-36, XP000602264 see abstract see table 1 -----	1-7,9-13